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CONTENTS

Some natural relationships among strains of tobacco mosaic virus
ALBERT SIEGEL AND SAM G. WILDMAN 277

Vector-virus relationships of pea-enation mosaic and the pea aphid Macrosiphum pisi (Kalt.) JOHN N. SIMONS 283

Mechanical and aphid transmission of the feathery mottle virus of sweet potato
R. E. WEBB AND R. H. LARSON 290

Relative pathogenicity of fungi associated with root rots of red clover in Wisconsin
R. A. KILPATRICK, E. W. HANSON, AND J. G. DICKSON 292

The infection of Nicotiana glutinosa with tobacco mosaic virus as affected by potassium nutrition WILLIAM B. ALLINGTON AND EDWARD F. LAIRD, JR. 297

Factors in the development of blue damage of Spanish peanuts DON C. NORTON 300

Cucumber as a plant host in stone fruit virus research
J. S. BOYLE, J. DUAIN MOORE, AND G. W. KEITT 303

Heterothallism in Phytophthora parasitica var. nicotianae
E. M. JOHNSON AND W. D. VALLEAU 312

Detection of virus in Cattleya orchids by serological methods
MILTON ZAITLIN, A. M. SCHECHTMAN, J. G. BALD, AND S. G. WILDMAN 314

A comparative study of Diaporthe stem canker and pod and stem blight of soybean
KIRK L. ATHOW AND RALPH M. CALDWELL 319

Effect of temperature on the development of downy mildew of lima bean R. S. COX 325

PHYTOPATHOLOGICAL NOTES:

Longevity of the oak wilt fungus in diseased trees
EDWARD L. MEREK AND CHARLES L. FERGUS 328

Inhibition of tobacco mosaic virus by an antibiotic from an actinomycete, Nocardia species DAVID E. SCHLEGEL AND T. E. RAWLINS 328

Soaking treatments for the control of loose smut of barley
CURT LEBEN AND D. C. ARNY 329

Report and abstracts of the 1954 annual meeting of the Southern Division of the American Phytopathological Society 331

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SOME NATURAL RELATIONSHIPS AMONG STRAINS OF TOBACCO MOSAIC VIRUS [1]

Albert Siegel [2] and Sam G. Wildman

SUMMARY

Strains of tobacco mosaic virus have been found to differ in two new ways, color and sensitivity to inactivation with ultraviolet light.

Eight strains of tobacco mosaic virus have been characterized as to symptoms in *Nicotiana tabacum*, color, sensitivity to inactivation with ultraviolet light, electrophoretic mobility, and reaction on *Nicotiana sylvestris*. These 8 strains fall into 4 natural groups. Within a group, strains can be distinguished only by their symptoms in *N. tabacum*, and are identical in all 4 other criteria applied. Two strains with identical symptoms in *N. tabacum* were found to differ in all 4 other characteristics.

A correlation has been found between the physical and chemical properties of a virus strain and one of its biological properties, the type of response elicited on *N. sylvestris*.

Several attempts have been made to correlate the biological behavior of various strains of tobacco mosaic virus (TMV) with their physical and chemical properties. Although strains of TMV have been found to differ in a number of characteristics such as electrophoretic mobility (6, 12, 14, 20), isoelectric point (16), serological properties (2, 4, 6, 15, 20), and amino acid composition (13), in no instance has it yet been possible to relate specifically any of these *in vitro* properties of the virus particles to biological activity. Differences have been found among the *in vitro* properties of TMV strains which have been considered to be derived from each other by mutation (6, 13), but the difficulty in deciding which strains are directly related to each other by a single mutational step complicates the interpretation of such experiments.

The present study attempts to correlate the *in vitro* properties of the virus with its biological activity. A systematic study of several physical-chemical and biological properties of 8 randomly selected strains of TMV has been undertaken as a collaborative venture by the members of this laboratory. This paper presents the initial findings that the 8 strains fall into 4 natural groups when compared on the basis of the following characteristics: type of symptom in *Nicotiana sylvestris*, electrophoretic mobility, sensitivity to inactivation with ultraviolet light, and color. Furthermore, differences among these strains suggest a series of markers suitable for investigation of the possibility that recombination occurs among plant viruses.

MATERIALS AND METHODS.—*Strains of tobacco mosaic virus.*—The 8 strains of TMV used in this investigation were obtained from various sources. To avoid confusion, they are designated simply as U1 . . . . . U8, the U indicating that the strains are being maintained as laboratory stocks at U.C.L.A., and the number indicating the order in which the strains were isolated or reisolated and purified. This nomenclature is not intended to supersede any previous attempts at classifying strains of TMV but has been introduced to refer specifically to our present laboratory strains and to differentiate these from stocks maintained in other laboratories which may or may not be different.

The source of the strains and the symptoms they produce when grown in *Nicotiana tabacum* var. Turkish Samsun are as follows:

The isolate from which U1 was obtained was received from the late Professor James Johnson of the University of Wisconsin as an example of common TMV. The virus was supplied in a dried infected leaf, and was purified by passage through a local lesion on *Nicotiana glutinosa* before it was grown in quantity in *N. tabacum*. The purified virus produces symptoms in *N. tabacum* typical in all respects of those described for common TMV.

U2 was found in the sample from which U1 was isolated (20). The virus produces a mild form of light green-dark green mosaic, a symptom which can be completely masked by the presence of common TMV. This strain when pure has never reverted to common TMV in spite of numerous transfers over a 3-year period.

U3 is a strain provided by Dr. C. A. Knight of the University of California at Berkeley. The virus was originally considered to be an example of Holmes' rib grass strain, but the symptoms which develop in *N. tabacum* are not typical of those described by Holmes (11) although the symptoms—dark green veinbanding with light green interveinal areas—are different from any of the other strains in our collection.

U4 was also obtained from Dr. Knight, and agrees in all respects with Holmes' description (10) of the masked strain of TMV.

U5 was isolated by Professor J. G. Bald of the University of California, Los Angeles. The virus was found in wild *Nicotiana glauca* plants where it produces a bright yellow mottle. In *N. tabacum*, this

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[2] Formerly a Public Health Service Postdoctoral Fellow of the National Institutes of Health, under the sponsorship of Professor Bald.

strain produces marked rugosity of the younger leaves followed by a slight mottle as the leaves mature.

U6 was isolated from a yellow area on a tobacco leaf systemically infected with U1. Symptoms of the virus consist of a bright yellow-dark green mosaic with a good deal of necrosis and marked stunting when young plants are infected.

U7 was isolated from a sample of U1 which had been irradiated with ultraviolet light so that only about 10 per cent of the original preparation remained infective. The treated virus was applied to *N. glutinosa* and the virus contained in single lesions was transferred to small *N. tabacum* plants. Among many plants which showed the typical symptoms of strain U1, a few had much milder symptoms. From 1 of these plants, the virus was isolated and multiplied in *N. tabacum*. U7 is indistinguishable from U2 in *N. tabacum*, but produces a systemic symptom in *Nicotiana sylvestris* in contrast to U2 which produces only local lesions on this host.

U8 was kindly supplied by Dr. L. O. Kunkel of the Rockefeller Institute as a sample of Holmes' rib grass strain. The symptoms produced in *N. tabacum* are typical of those described by Holmes (11).

All 8 strains used in this study were reisolated from single *N. glutinosa* lesions before being multiplied and isolated in quantity from *N. tabacum* plants. In our experience, maximum yields of virus are obtained when well-expanded leaves are directly inoculated, and the virus allowed to multiply for about 18 days.

Virus purification method.—Equal parts by weight of infected leaves, from which the midribs had been removed, were ground with equal parts by volume of 0.1 ionic strength cacodylic acid buffer, pH 6.9. A colloid mill was used to grind leaf samples greater than 100 gm. fresh weight and an Omnimixer type of blendor was used for smaller leaf samples. The slurry was passed through a basket centrifuge lined with sharkskin filter paper to remove cell wall residues. The filtrate was frozen and 24 hours later, thawed to facilitate aggregation of the green matter. If the leaves are frozen and thawed prior to grinding, this step may be eliminated. The green matter was removed as a sediment by centrifuging the filtrate for 20 minutes at 8000 g.

The virus was purified by 3 or more cycles of alternate high (30,000 r.p.m., Spinco model L, #30 rotor) and low speed (8000 g.) centrifugation until the virus pellets deposited by high speed were completely clear when observed by transmitted light, and the supernatant solutions were colorless. Generally, 3 cycles of centrifugation were sufficient to achieve this condition.

Ultraviolet irradiation.—The strains, diluted at least 100-fold into 0.067 M phosphate buffer, pH 7, were irradiated in a 1–2 mm. layer of known concentration at a distance of 24.5 cm. from a Westinghouse Sterilamp, Model 782L-30, which has an output mainly at 2537 Å.

Local lesion bioassay procedure.—The infectivities of the virus preparations were determined by the local lesion half-leaf method (19), using *Nicotiana glutinosa* as the test plant. Care was taken to insure that the activities fell in a region of the assay curve where the number of lesions was approximately proportional to the concentration of virus applied. The experimental scheme most commonly employed was an incomplete block design (21) arranged to minimize the variability inherent in different plants, the leaf level on individual plants, and the rubbing pressure on different halves of the leaf. Thirteen different treatments were compared in an experiment on 26 uniform plants, each containing 6 well expanded leaves. Each treatment appeared with each of the other 12 treatments on opposite halves of a leaf. The scheme allows for 24 replicates of each treatment. Each treatment appeared no more than once per plant and was omitted from 2 plants. The replicates were distributed equally among the 6 leaf levels and appeared an equal number of times on left and right half-leaves. While extreme variation in the number of lesions arising per half-leaf was always encountered, the total number of lesions which arose from 24 half-leaves usually agreed within 5 per cent with the total from a duplicate treatment in the same experiment, and only very rarely differed by more than 10 per cent.

Electrophoretic analyses. — Electrophoresis experiments were performed in a Pearson type of apparatus employing cells of 11 and 2.8 ml. capacity. All analyses reported in this paper were performed in 0.1 ionic strength cacodylic acid buffer, pH 6.9 containing 0.08M NaCl, 0.02M Na cacodylate, and 0.0033M cacodylic acid. Current was applied to create a potential gradient of 4.42 volts/cm. in the large cells, and 3.18 volts/cm. in the small cells. Usually, a migration period of 3600 seconds was used but this period was frequently extended.

EXPERIMENTAL RESULTS.—*Color of the virus preparations.*—When isolated and purified according to the method described above, preparations of 5 of the 8 strains of TMV used in this study were found to have a brown or an amber color which was made evident when the viruses were compacted into a pellet by centrifuging or maintained as concentrated suspensions containing 1 per cent or more virus. In contrast, preparations of 3 of the strains were completely lacking in color; the pellets were clear, and concentrated suspensions appeared milky white. As shown in Table 2, preparations of strains U1, U3, U4, U6, and U7 were brown, while strains U2, U5, and U8 were colorless.

Ginoza et al (7) have shown that the brown color results from combination of a host nucleoprotein with the virus. This nucleoprotein can be removed from the virus by a variety of reagents, leaving the "brown" strains colorless. The decolorized virus will readsorb the host nucleoprotein *in vitro* under appropriate conditions. Strains U2, U5, and U8 have never appeared colored upon extraction and will not adsorb the host nucleoprotein *in vitro*. Thus, the difference between the colored and colorless preparations resides in the

TABLE 1.—*The inactivation of strains of TMV by ultraviolet light*

Dose Min.	No. of lesions on 24 half-leaves			Relative number of lesions		
	U1	U8	U2	U1	U8	U2
0	1230 1277	1007 914	1541 1731	100	100	100
1		608	292		63	18
3	754	147	11	60	15	0.7
6	271	28		22	3	

ability of the strains to complex with a host nucleoprotein. It is necessary to stress the importance of using cacodylic acid, or some other monovalent buffer, in the isolation procedure to detect the difference between the 2 types of strains. Since phosphate ions will remove the brown color from the colored preparations, the use of a buffer system containing phosphate ions may provide ambiguous results in regard to the color characteristics of the strains.

The brown color does not arise from the cultural conditions under which the plants and virus strains are grown, since virus pellets of equal color intensity have been obtained repeatedly, even though plants have been grown at all times of the year under quite different conditions of light intensity and temperature. The color is also not associated with the host plant in which the virus strain is grown, since U1 has been recovered from tomato, *N. sylvestris*, and *N. tabacum* var. Havana 38, in each instance giving the same type of colored pellet and suspension as when grown in Turkish tobacco. Similarly, U2 when grown in different hosts is recovered in a colorless condition.

Difference in sensitivity of strains of TMV to inactivation by ultraviolet light.—Previous work (1, 9, 17, 18) on the sensitivity of TMV to inactivation with UV has been concerned only with the behavior of the common strain. We have observed that strains of TMV differ greatly in this respect. Table 1 presents the data of an experiment in which 3 strains of different sensitivities were compared. Samples of strains U1, U8, and U2 were irradiated with UV for various lengths of time at a concentration of 10^{-3} mg. of virus per ml. Two determinations were made of the nonirradiated control samples and it can be seen that very reasonable duplications of the number of lesions on 24 half-leaves was obtained. Consequently, results of the bioassay of the irradiated samples may be considered reliable. On the right side of Table 1 the relative number of lesions given by the 3 strains after exposure to UV is listed. After a dose of 3 minutes, less than 1 per cent of the initial virus activity of strain U2 was recovered, whereas 15 per cent of the activity of U8, and 60 per cent of U1 survived this treatment. It is therefore apparent that TMV strains are markedly different in their susceptibility to inactivation by UV. When the remaining 5 strains were examined, it was found that strains U3, U4, U6, and U7 are similar in sensitivity to U1, and strain U5 is similar to U2. Results of many experiments are plotted in Fig. 1. In agreement with others (1, 17, 18), the course of inactivation of virus activity with increasing dose of irradiation falls on a straight line when plotted on a semi-logarithmic scale. The eight strains fall into 3 distinct classes: 1) Strains U1, U3, U4, U6, and U7 are relatively resistant to inactivation; 2) Strains U2 and U5 are highly susceptible to inactivation; 3) Strain U8 is intermediate in sensitivity.

There is a possibility that small differences in sensitivity may exist among the strains in the above classes 1 or 2. However, any such differences would be small compared with the large differences found among these classes of strains.

Preparations of those strains which are resistant are colored, whereas preparations of those which are intermediate and sensitive are colorless. The possibility was tested that the brown color acts as a protective agent and thereby causes the "colored" strains to appear more resistant to UV inactivation than the "colorless" strains. U1 was decolorized by the method of Ginoza et al (7). After decolorization the virus was

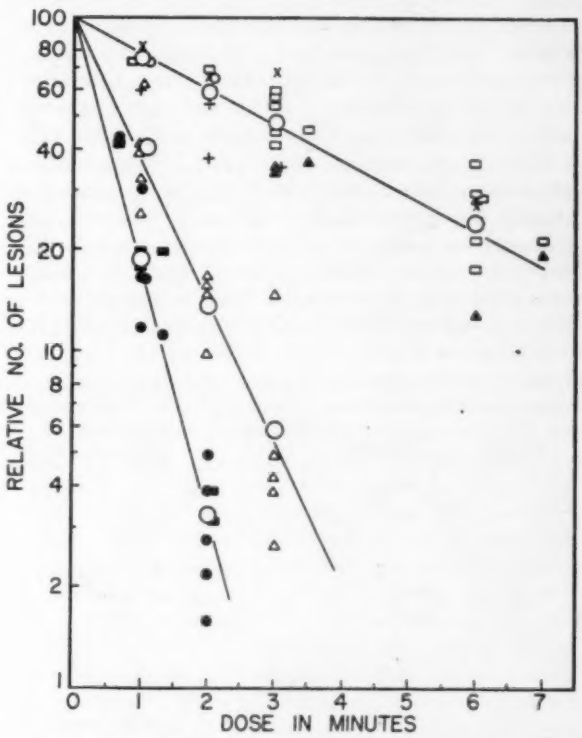

FIG. 1. Sensitivity of the 8 strains to inactivation with UV. The results of several experiments are plotted on this graph. The large open circles represent the average of all values obtained at the indicated dose for strains with the same sensitivity. The upper curve represents the sensitivity of the resistant strains; the middle curve is for the intermediate strain; the lower curve represents the 2 sensitive strains. The symbols for the individual strains are: □ U1, ■ U2, ○ U3, + U4, ● U5, × U6, ▲ U7, △ U8.

found to be inactivated in precisely the same manner as "colored" U1. Evidently, the brown color does not contribute to the differences in the UV sensitivities of the strains.

The resistant strains require about 7 times the dose to inactivate a given proportion of the virus as do the sensitive strains, and they are also about 4 times more resistant than the intermediate strain. Oster and McLaren (17) have calculated that 23,300 quanta are required for the inactivation of a virus particle of the common strain. If our common strain, U1, is identical with theirs, then strains U2 and U5 require only 3,300 quanta per inactivation, while strain U8 requires 5,800.

Electrophoretic mobility.—The electrophoretic mobilities of the 8 strains were determined at pH 6.9 under the standard conditions previously described (Table 2). Strains U1, U3, U4, and U7 have a mobility of -8.9×10^{-5} cm./sec./volt/cm. Strains U2 and U5 have a mobility of -4.9×10^{-5}. Strain U6 has a mobility of -6.8×10^{-5} and for U8 the value is -7.7×10^{-5}. The mobilities reported here for strains U1 and U2 do not agree precisely with those given by Singer et al (20), who found the values of -7.8 and -4.8×10^{-5}, respectively, for these 2 strains under similar conditions of analysis. The reason for this discrepancy is unknown at the present time. Our values for U1 and U8 [Holmes' rib grass strain], however, agree with those reported by Knight and Lauffer (12).

The strains reported to have the same electrophoretic mobility were tested for the possibility of slightly different mobilities, differences that might be obscured by errors in determining the value for any individual strain. Each of the strains reported to have a mobility of -8.9×10^{-5} was mixed with strain U1 in equal proportions and analyzed in the Tiselius apparatus. In no case was more than 1 boundary established after a 2-hour migration time, indicating that these strains have the same electrophoretic mobility under these conditions. The same result was obtained with a mixture of strains U2 and U5 which have the slower mobility of -4.9×10^{-5}.

The strains reported to have the same mobility at pH 7 were found by Ginoza[3] to have identical mobilities regardless of pH. The mobilities at different pHs vary from -4.3 to -8.9×10^{-5} for strains U1, U3, U4, and U7, and from -2.8 to -4.9×10^{-5} for strains U2 and U5.

When U1 is decolorized, it has the same mobility as colored U1 (7), demonstrating that the different mobilities for the various strains cannot be attributed to the fact that some strains are colored while others are not.

Reaction of Nicotiana sylvestris to inoculation with the eight strains.—Certain strains of TMV invade *N. sylvestris* systemically and other strains cause formation of necrotic local lesions on inoculated leaves of this host. Strains U1, U3, U4, and U7 invade *N. sylvestris* systemically (Table 2). Strains U2, U5, U6 and U8 elicit the necrotic local lesion response in this host and fail to become systemic.

Discussion.—Data obtained in this study are in Table 2 where it can be seen that the 8 strains examined fall into 4 natural groups. Within a group, strains can be distinguished only by their symptoms in *N. tabacum*, and are identical in the 4 other properties that have been investigated.

Group 1, which includes U1 (common TMV), contains also 3 other strains, U3, U4, and U7. While each of these strains produces different symptoms in *N.*

[3] Ginoza, W. personal communication.

Table 2.—*Classification of 8 strains of TMV on the basis of 2 biological and 3 physical-chemical characteristics*

Group	Strain	Common name	Symptoms in *N. tabacum*	Color	Electrophoretic Mobility $\times 10^{-5}$	Relative UV Sensitivity	Reaction on *N. sylvestris*
1	U1	severe, common	typical mosaic	brown	—8.9	resistant	systemic
	U3		dark green veinbanding	brown	—8.9	resistant	systemic
	U4	masked	none	brown	—8.9	resistant	systemic
	U7		mild mosaic	brown	—8.9	resistant	systemic
3	U6		yellow mosaic	brown	—6.8	resistant	local lesion
4	U8	Holmes' Rib Grass	necrotic spots and bands on leaves	colorless	—7.7	intermediate	local lesion
2	U2	mild	mild mosaic	colorless	—4.9	sensitive	local lesion
	U5		rugose leaves	colorless	—4.9	sensitive	local lesion

tabacum, none of the 4 can be distinguished from the others by any of their other 4 properties. Knight (13) has found U1 and U4 to be the only strains of identical amino acid composition, of those he has analyzed.

Group 2 consists of 2 strains, U5 and U2, which differ from the Group 1 strains in all the properties investigated except symptoms in *N. tabacum*. Although the 2 strains are identical in the physical properties examined and in reaction on *N. sylvestris*, they are readily distinguished from each other by the symptoms they produce in *N. tabacum*.

Group 3 consists of 1 strain, U6, which is different from Group 1 strains in 2 of 4 properties—electrophoretic mobility and reaction on *N. sylvestris*.

Group 4 consists of 1 strain, U8, which has no investigated properties identical with strains of Group 1, and differs in 2 respects—mobility and sensitivity to UV—from strains of Group 2. Amino acid analyses performed by Knight (13) also suggest that U8 is not closely related to Group 1 strains since U8 possesses 2 amino acids not found in U1 besides other marked differences in composition.

The groups have been numbered in the order of their discovery and are arranged in Table 2 to indicate their presumed degree of relatedness. Thus, strains in Groups 1 and 2 are considered to be the least related to each other. Group 3 is considered to be more related to Group 1 than to Group 2, whereas Group 4 appears to be more closely related to Group 2 than to Group 1. This natural classification has been verified by the serological investigations of Dr. Irving Rappaport.[4]

A striking aspect of this investigation is the finding that 2 strains, U2 and U7, exhibit the same symptom expression in Turkish tobacco and yet are grossly different from each other in each of the 4 other physical-chemical and biological properties examined. Evidently symptom expression by itself is not an adequate means of classifying strains on the basis of their degree of relatedness.

The question may be raised as to what type of subtle differences exist among the 4 strains of Group 1 to account for the different symptom pattern that each produces in *N. tabacum*. One explanation is that these strains differ only in that portion of the virus nucleoprotein molecule which is responsible for the genetical continuity of the virus, and that this vital portion may constitute only a small part of the whole virus particle as it is isolated. Hershey and Chase (7) have recently described a case in which only a portion of a bacterial virus is responsible for its genetic continuity. Future work may well show that a large part of what is isolated as purified plant virus nucleoprotein is unnecessary for the essential *in vivo* reproductive activities of the virus.

At the present time, it appears difficult to implicate any of the *in vitro* properties of TMV with the type of symptom produced in *N. tabacum*, for we see that strains lacking notable differences in the *in vitro* properties studied produce grossly different symptoms. Contrariwise, strains which produce the same symptom in *N. tabacum* may differ in all 4 other properties investigated. On the basis of this evidence, it may appear a hopeless task to correlate the physical-chemical structure of the virus with its biological activity. However, a remarkable correlation does appear among the 8 strains in that strains of Group 1 which are able to invade *N. sylvestris* systemically possess the same electrophoretic mobility, sensitivity to inactivation with UV, and color. In marked contrast, a strain which differs in 1 or more of these properties is confined to a local lesion on this host, as in the case of strain U6 which has a slower electrophoretic mobility than the group 1 strains. Our present information therefore indicates that as far as these eight strains are concerned, only those with a certain specific set of *in vitro* properties can accomplish the systemic invasion of *N. sylvestris*. Consequently, it would seem that a reasonable prospect exists for reducing biological specificity in this system to the level where specific chemical configurations of the virus molecule can be implicated in certain types of biological activity.

The question arises as to how one may classify strains of TMV on a phylogenetic basis; such a classification is clearly demanded for the study of the effects of a specific mutation on alterations in the chemical structure of the virus and in its genic material. The practice has long been prevalent of calling strains of TMV derived from a parent strain, mutants, with the implication by some investigators that such variants had arisen by a single mutational step. In addition to single-step mutation, there are also other processes which could lead to the appearance of the variants isolated, particularly when it is considered that upwards of 10^{14} virus particles can be extracted from a single infected leaf of *N. tabacum*. Under the conditions existing in such a vast population, it is to be expected that in most cases only those variants which can compete at all successfully with the original parent strain will be present in numbers great enough to be detected. Thus there is no reason to suppose that such a variant is necessarily related to the parent strain by a single mutational step. Indeed, many mutational steps may have intervened until a form had arisen which could multiply to the extent that it could be detected by an observer. Another mechanism which could account for the appearance of competitively successful variants of TMV is mutation followed by recombination. This phenomenon has been shown to occur in certain of the bacterial (5) and animal viruses (3). In the absence of more precise information as to the genetic behavior of TMV it is difficult to speculate on this as well as the other postulated modes of origin of the many strains of TMV.

The establishment of a series of strains with several sorts of differences among the individual variants has provided us with a system which will be useful in an investigation to discover whether or not TMV under-

[4] Personal communication.

goes recombination. Such an investigation should reveal whether this virus is polygenic or monogenic. This information will better define the TMV experimental system and will have obvious implications for the several basic biological problems for which investigators have found TMV to be such a promising tool.

We have already pointed out that TMV has been fairly well-defined chemically and there is no doubt that with further study this biological entity can become better known in a chemical sense. TMV also shows a remarkable degree of variation. In the past much stress has been placed on the chemical characterization of this virus and on the chemical differences which exist among strains, while relatively little attention has been focused on the genetic basis of the observed variation. The study of the physical and chemical basis for biological variation is clearly dependent not only upon a definition of chemical differences among strains of TMV but is also necessarily dependent upon a clearer understanding of the biological origin of the various strains.

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VECTOR-VIRUS RELATIONSHIPS OF PEA-ENATION MOSAIC AND THE PEA APHID MACROSIPHUM PISI (KALT.) [1]

John N. Simons [2]

SUMMARY

Pea-enation mosaic virus was transmitted experimentally by the pea aphid, *Macrosiphum pisi* (Kalt.); the potato aphid, *M. solanifolii* (Ashm.) (11); the green peach aphid, *Myzus persicae* (Sulz.); and the ornate aphid, *M. ornatus* (Laing). An experimental host range which included garden pea, sweet pea, bur clover, spotted Medick, crimson clover, and soy bean was demonstrated.

The acquisition threshold period for adult pea aphids was found to lie between 1 and 2 hours; the inoculation threshold period was between 15 and 20 minutes in length. The effect of length of acquisition feeding on rate of pickup was almost linear; the effect of length of test feeding on rate of transmission was logarithmic.

Evidence presented indicated a positive correlation between length of acquisition feeding and length of retention of virus. Post-acquisition feeding starvation for periods up to 24 hours produced no effect on transmission.

First instar pea aphid nymphs were more efficient vectors than adult pea aphids. The nymphs showed a shorter mean latent period (30.0 hours) than the adults (56.8 hours) and it was proposed that this might be a reflection of differences in vector efficiency. The presence of a discernible latent period for every vector of this virus was questioned. Positive evidence was presented that the latent period, where demonstrable, can be completed on the source plant.

Pea-enation mosaic is one of the most serious diseases of garden pea (*Pisum sativum* L.). Three species of aphids, the pea aphid, *Macrosiphum pisi* (Kalt.) (7); the potato aphid, *M. solanifolii* (Ashm.) (7); and the green peach aphid, *Myzus persicae* (Sulz.) (4) have been shown to be experimental vectors. Because the virus causing this disease is retained in its insect vector for many days after having been acquired, it is placed in the persistent group of aphid-borne viruses (18). It is somewhat unusual among this group of plant viruses, however, in being at times readily transmissible by mechanical inoculation.

REVIEW OF LITERATURE.—The symptoms of pea-enation mosaic on many of its host plants were first described by Osborn (7). He found that the virus was transmitted by the pea aphid and the potato aphid. In addition he demonstrated that it could be retained in its vector for as long as 29 days and that a latent period of at least 4 hours was associated with the vector. Chaudhuri (4) was the first to report the green peach aphid as a vector.

Although not all persistent aphid-borne viruses have been shown to possess definite latent periods in their vectors, pea-enation mosaic appears to belong in the group requiring a latent period in the vector before successful transmission can be accomplished. The first of this group of viruses reported to have a latent period was potato leafroll (5, 11). Others include red-leaf of filaree (1) and strawberry severe crinkle (9). Anderson (1) was the first to call attention to a difference in the time required to pass the latent period between nymphal and adult forms of aphids.

MATERIALS AND METHODS.—The original source of virus was a naturally infected sweet pea plant growing in Berkeley, California. All subsequent sources of virus used in the experimental work were sub-inoculations from this plant.

Bur clover, *Medicago hispida* Gaertn., was used throughout the work both as a source of virus and as a test plant. The bur clover was germinated in seed pans of sterile sand and then transplanted to 3 in. clay pots. The age of the test plants used varied from 10-18 days. Leaf development of these plants ranged from the cotyledon stage to seedlings where the first true leaf had unfolded.

The principal vector used during the work was the pea aphid, *Macrosiphum pisi* (Kalt.). Noninfective colonies were maintained on large bur clover plants and originated from offspring of insects taken on the sweet pea plant used as the original source of virus.

All aphid transfers were made by the camel's hair brush technique. The aphids were caged during the test feeding periods by means of 32 × 90 mm. glass shell vials. Following the completion of the test feeding periods all plants were either fumigated with nicotine or sprayed with insecticide before being placed in the greenhouse.

HOST RANGE.—Because of the failure to obtain consistent transmission of the virus by mechanical inoculation techniques, the host range was determined by using pea aphids which had been reared on diseased bur clover plants. Five aphids per plant were used and 25 plants of each species were inoculated. The following species of plants, all in the family Leguminosae, were experimentally infected: garden pea, varieties Alaska and Laxton Progress (*Pisum sativum*

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L.); sweet pea, var. Early Flowering Spencer (*Lathyrus odoratus* L.); soy bean (*Soya max* L.); crimson clover (*Trifolium incarnatum* L.); and bur clover (*Medicago hispida* Gaertn.). In addition, spotted Medick, *Medicago arabica* All. was found to be a naturally infected host plant.

The following species of plants failed to develop symptoms: CHENOPODIACEAE: sugar beet (*Beta vulgaris* L.); CRUCIFERAE: smooth leaf mustard (*Brassica juncea* Coss); CUCURBITACEAE: cucumber (*Cucumis sativus* L.); LEGUMINOSAE: broad bean (*Vicia faba* L.); garden bean, varieties Robust and Stringless Refugee (*Phaseolus vulgaris* L.); alfalfa (*Medicago sativa* L.); spring vetch (*Vicia villosa* L.); white clover (*Trifolium repens* L.); alsike clover (*T. hybridum* L.); red clover (*T. pratense* L.); and ladino clover, polyploid variety of *T. repens* L.; SOLANACEAE: tomato, var. Marglobe (*Lycopersicon esculentum* L.); and tobacco, var. Turkish (*Nicotiana tabacum* L.); UMBELLIFERAES celery (*Apium graveolens* L. var. *dulce*).

Symptoms.—The first symptom of pea-enation mosaic in bur clover seedlings is usually a yellowing of the main veins of the youngest leaf. Later the veinlets become mostly yellow, the leaflets puckered and deformed; occasionally small reddish-yellow necrotic spots appear. Often the plant becomes severely stunted. In very young seedlings the first symptom may be an abrupt bending down of the petiole of the first true leaf.

AVAILABILITY THRESHOLD.—The term availability threshold denotes the time following the inoculation of a plant that intervenes before virus becomes available to a previously noninfective vector. The procedure used in determining this availability threshold was as follows. Ten healthy bur clover plants were each infested with 3 infective adult pea aphids which had received access feedings of 70 hours. Five individual nymphs from each plant were transferred daily to each of 5 healthy bur clover seedlings for 6 days and were then given test feedings of 72 hours. At the end of 6 days the source plants were fumigated and placed in the greenhouse with the test plants. Results indicated that virus can become available to a vector several days before symptoms appear. Virus became available in 1 plant 5 days after inoculation and in 3 other plants 6 days after inoculation. Symptoms in these plants were not evident for 9 days in the case of 1 test, 10 days in 2 other tests, and 13 days in the fourth. The mean time in which virus was recovered was 5.8 days.

APHID VECTORS.—Three species of aphids, the pea aphid, *Macrosiphum pisi* (Kalt.); the potato aphid *Macrosiphum solanifolii* (Ashm.); and the green peach aphid, *Myzus persicae* (Sulz.) have been demonstrated to be vectors. The bean aphid, *Aphis rumicis* L., has been reported by Osborn (7) as not being a vector. In the following trials the first 3 of the above named species as well as the cotton aphid, *Aphis gossypii* Glover; the bur clover aphid, *A. medicaginis* Koch; the foxglove aphid, *Myzus solani* (Kalt.); and the ornate aphid, *M. ornatus* (Laing) were tested.

The access feeding period for each of the species was approximately 48 hours and the test feeding period was 72 hours. Twenty-five bur clover seedlings were inoculated by each species tested. Five aphids per plant were used except in the cases of the pea aphid, the potato aphid, and the green peach aphid, for which only 3 individuals per plant were utilized. Check colonies of adult pea aphids were fed with each species except for the potato aphid and the green peach aphid in order to verify the source of inoculum. The pea aphid infected 19 of 25 plants, the green peach aphid infected 9 of 25, the potato aphid infected 17 of 25, and the ornate aphid transmitted to 15 of the 25 plants tested.

ACQUISITION THRESHOLD PERIOD.—In determining the acquisition threshold period for pea-enation mosaic virus the following procedure was used. Noninfective adult pea aphids were placed on an infected bur clover plant and were given acquisition (watched feedings) of 25 seconds and 5 minutes as well as access (unwatched feedings) of 15, 30, 60, and 120 minutes. As soon as the aphids had completed their feedings they were placed singly on bur clover seedlings for test feeding periods of from 84–91 hours. Three replications using groups of 10 individuals at each of the 6 feeding times were done. Because only 1 infection (at the 2-hour level) resulted from this series of tests an additional trial using 15 insects at each of 3 access times, 60, 120, and 180 minutes was carried out. One infection at the 3 hour level was obtained.

Results indicated that the virus could be acquired in a feeding period of between 1 and 2 hours Since the disease can occasionally be transmitted by mechanical inoculation, the virus may be present in the mesophyll tissue and not restricted to the phloem regions. Consequently a much shorter acquisition threshold period might be expected. One possible explanation for this long acquisition threshold period is that the titre of virus in the plant is so low that the insect vector has only a slight probability of picking up an infective charge during a short feeding time.

Effect of length of access feeding on efficiency of transmission.—Preliminary work with small groups of insects reared on diseased plants indicated that vector efficiency was high if the aphids were given long periods of access feeding. Since the amount of transmission by aphids which had received acquisition or access feedings of less than 4 hours seemed considerably lower, it was decided to determine at what rate virus was acquired during the course of a several day access feeding period.

Large numbers of noninfective adult pea aphids were placed on 2 bur clover source plants. Insects were then removed at the end of access feeding times of 2, 4, 8, 12, 24, and 48 hours. Two replications of 10 plants per time interval and 1 aphid per plant for each of the 2 sources were run. In addition, 1 repli-

cation using each of these same sources and treatments similar to those above except for the extension of the access feeding periods to include 72 and 120 hour intervals was carried out. In all, 60 plants at each of the 2, 4, 8, 12, 24, and 48 hour feeding periods and 30 plants at the 72 and 120 hour intervals were tested. These insects were given test feeding periods of 3 days for the 2–48 hour periods and 1 day for the 72 and 120 hour intervals.

Results indicated that the pickup rate was approximately directly proportional to the length of time spent feeding on inoculum (Fig. 1). No infections were obtained at the 2 and 4 hour levels whereas 3 of 60 aphids tested were vectors at the 8-hour period, 1 of 60 at 12 hours, 7 of 60 at 24 hours, 15 of 60 at 48 hours, 6 of 30 at 72 hours, and 15 of 30 proved infective after a 120 hour access feeding. Results similar to these were obtained by Watson (17) while working with beet yellows virus. They probably indicate 2 things: 1) virus occurs evenly distributed in that area of the plant in which the aphid feeds, and 2) the aphids feed at a relatively constant rate.

Effect of length of access feeding on retention.—Osborn (7) showed that pea-enation mosaic virus could be retained in the pea aphid for periods up to 29 days. His tables, in the case of individual aphids, also indicated that as transmission continued over several weeks of daily transfers, there were considerably more infections present in the first 10 days of transfers than in the following 10 days of a series. In the case of at least 3 other virus diseases, curly top of sugar beet (6), best yellows (17), and Stubb's carrot disease (12), a correlation between length of retention and length of the access feeding period has been shown. In all 3 cases, longer periods of access feeding gave increased retention time. To demonstrate a similar phenomenon for pea-enation mosaic the following series of tests was carried out.

First instar pea aphid nymphs (ave. age 12 hrs.) were reared on healthy bur clover plants to the adult form, and 50 were then placed on a bur clover inoculum plant for access feedings of 24, 48, and 96 hours. Ten aphids were placed singly on bur clover test plants and transferred daily (except for the 24-hour group which was allowed to feed for 2 days on the first plant) until all the insects were dead. Three replications, all of which utilized a common virus source, were carried out.

Retention times for insects which lived at least 19 days after completing the access feeding period were as follows: 24-hour group ranged from 6–15 days (mean 11.0 days), 48-hour group ranged from 8–23 days (mean 14.6 days), and the 96-hour group ranged from 9–21 days (mean 15.0 days). The records of these groups of vectors is presented in graphic form in Fig. 2. The negative correlation between mortality and length of retention is evident. The number of transmissions obtained in the first 10 days of serial transfer was much greater than during the eleventh to twentieth day regardless of the length of access feeding used. For example vectors which had received a 24-hour access feeding made 13 transmissions during the first 10 days of transfer and 5 during the following 10 days, a ratio of 13:5. In the 48-hour group the ratio of transmissions over the 2 10-day periods was 22:12 and in the 96-hour series this ratio was 47:21. These results suggest a definite loss of virus over a daily serial transfer period of 20 days and failure of the virus to multiply in the vectors.

It seems likely that a vector which is able to retain virus for several weeks should begin with a higher titre of virus than one which transmits for a shorter period of time. This higher charge of virus should be reflected in a test of the above type by a greater number of plants being infected per day of serial transfer by the vector which retains the ability to infect for a longer period. To appraise this hypothesis, the mean number of infections per day for each vector was calculated (total number of infections over a given number of days of transfer divided by the number of vectors) for the period between the fifth and eleventh

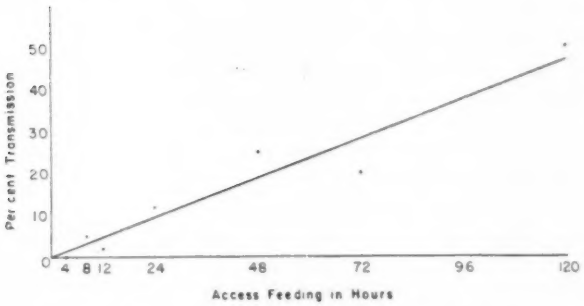

Fig. 1. Regression line, assumed linear, of percentage of transmission on length of access feeding. Y .1 .392X.

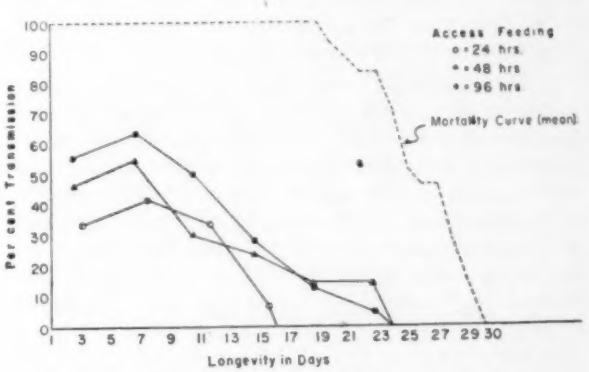

Fig. 2. Effect of 3 different access feeding periods on retention of pea-enation mosaic virus by adult pea aphids. Only records of vectors that lived at least 19 days are plotted. The mortality curve is a mean composite using the data from all the groups.

day after the beginning of the access feeding period. Results were as follows: 24-hour group = 0.44 infections per vector per day, 48-hour group = 0.52 infections per vector per day, and the 96-hour group = 0.61 infections per vector per day. These ratios indicate that insects allowed an access feeding period of 96 hours pick up more virus than insects which have received a 24-hour access feeding. The 96-hour vectors retained the ability to transmit for a longer period of time than did the 24-hour vectors.

Because only 4 vectors were obtained at the 24-hour level of access feeding, the experiment was repeated to test the above hypothesis more fully. The technique was the same as in the original except that the 48-hour feeding time was eliminated. The number of insects used at the 24-hour feeding was doubled. Thus 60 aphids, 20 per replication, were given 24-hour access feedings and 30 others, 10 per replication, were given 96-hour feedings.

Unfortunately, the insects in this second trial did not live so long on the average as did those in the original trials. However, 5 vectors of the 24-hour group and 3 vectors from the 96-hour group lived 19 days or longer. Retention times were as follows: 24-hour group ranged from 7–18 days (mean = 12.2 days), and the 96-hour group ranged from 11–22 days (mean = 17.3 days). Pooling the results of both experiments gives mean retention times of 11.7 (S.D. = ±4.08 days) for the 24-hour group and 15.6 (S.D. = ±4.70 days) for the 96-hour group. These additional data, while again not wholly satisfactory, support the idea that there is a positive correlation between the length of access feeding time and the length of virus retention in the vector.

INOCULATION THRESHOLD PERIOD.—To determine the inoculation threshold period a large number of noninfective adult pea aphids were placed on a bur clover source plant and given an access feeding of approximately 88 hours. Individuals from this colony were given test feedings ranging from 25 seconds to 4 hours. In all, 4 replications using at least 10 individuals for each feeding interval were carried out. In 1 replication 20 aphids instead of the usual 10 were used at the 30-minute interval.

No transmissions were obtained before a feeding period of 20 minutes had elapsed, and once this time had passed the amount of transmission increased exponentially (Fig. 3). These results indicate the presence of some sort of barrier to successful inoculation in feeding times of less than 20 minutes. The most obvious explanation is that the virus must be placed in the phloem before transmission can be accomplished. Sylvester (15) found that the inoculation threshold period of beet yellow-net was about 15 minutes and Anderson (1) working with filaree red-leaf obtained similar results. Since Roberts (10) had shown that aphids could reach phloem tissue in from 15–30 minutes of feeding time, both of the above workers felt that the phloem was the primary tissue in which successful transmission could be accomplished.

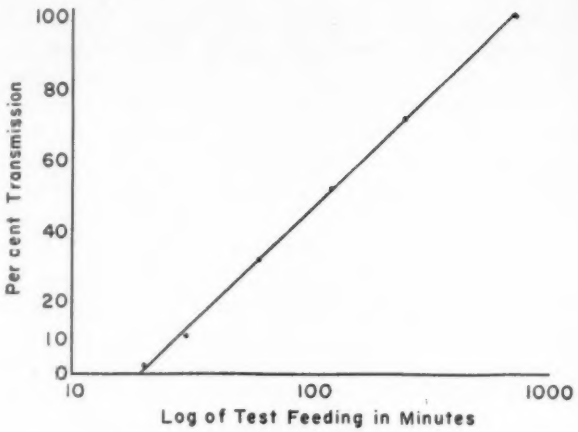

FIG. 3. Effect of length of test feeding period on transmission of pea-enation mosaic virus using adult pea aphids.

The fact that pea-enation mosaic can be transmitted mechanically gives some reason for doubting the above hypothesis. One possible explanation of this apparent anomaly is that the aphid may fail to release virus until its stylets have made contact with the phloem. If this is true then time required to reach the phloem would not be the barrier to successful transmission except in a secondary sense, the primary cause being tied up with the aphid's mechanism of feeding. If the above suggestion is true then there should be no significant lowering of this period even in the case of a juice-inoculable strain of the virus.

Another matter of importance shown by this experiment is that the latent period can definitely be passed on the source plant. Although the results of Chaudhuri (4) do not agree with these findings, the author feels that the positive evidence presented here conclusively demonstrates that pea-enation mosaic virus is not unique in having the latent period correlated with the vector's being either on or off the source of inoculum. Why Chaudhuri failed to establish this point is not clear, but the obvious explanation is that he failed to use sufficiently long access feeding times. In addition, his use of short initial test feeding periods may have tended to bias the results.

LATENT PERIOD IN ADULT AND NYMPHAL PEA APHIDS.—Osborn (7) was the first to demonstrate the presence of a latent period for the pea-enation mosaic virus in the pea aphid. Using an access feeding time of 8 hours and colonies of 50 adult aphids, his work indicated the presence of a minimum latent period of not less than 24 hours nor more than 28 hours in length. Chaudhuri (4) found a minimum latent period of between 6 and 26 hours.

Preliminary experiments by the author had indicated that a minimum latent period of between 28 and 48 hours existed in the adult pea aphid. To test this point further, groups of adult pea aphids were placed on 2 bur clover source plants and given access feed-

TABLE. 1—*Results*[a] *of trials to determine the latent period of pea-enation mosaic virus in adult pea aphids tested in groups of threes after receiving access feedings of 22 hrs.*[b]

Aphid lot no.	Total test feeding time in hours plus 21 hours of access feeding time				
	25	29	34	46	70
1	—	—	—	—	+
2	—	—	—	—	+
3	—	—	—	—	+
4	—	—	—	—	+
5	—	—	—	+	+
6	—	—	—	+	+
7	—	—	—	+	+
8	—	+	—	—	+
Total	0	1	0	3	8

[a] Plus (+) sign indicates plant was infected; minus (—) sign signifies plant remained healthy.
[b] Thirty groups were tested. Test feeding times were 4, 4, 5, 12, and 24 hours. Thirty groups were used.

ings of 22 hours. These were then divided into 30 lots of 3 insects each and moved serially to 5 successive healthy test plants. The test feeding periods used were 4 hours on the first plant, 5 hours each on the second and third plants, 12 hours on the fourth, and 24 hours on the fifth plant. All the aphids were alive at the end of the experiment (Table 1).

Eight of the 30 lots proved infective. There were no transmissions during the first 4-hour feeding period. One group transmitted during the 25-30 hour period, 3 groups became vectors during the 35-47 hour interval, and 5 colonies transmitted for the first time during the 47-71 hour feeding interval.

Anderson (1) working with filaree red-leaf virus, was the first to report a difference in the length of the latent period between nymphs and adults in an aphid-transmitted virus. He also noted that Osborn's tables (7) indicated that a similar phenomenon might exist in pea-enation mosaic transmission. The following tests were made to test the nymphal latent period.

A large number of first instar pea aphid nymphs (ave. age = 8.5 hr.) were placed on the same 2 source plants that were used in the latent period work on the adults. After access feedings of 22 hours the nymphs were moved in lots of threes to 30 test plants, 15 lots for each source. They were then moved serially to 5 successive test plants for test feeding periods of 4, 4, 5, 12, and 24 hours respectively. All the groups which proved infective were alive at the end of the test feeding periods (Table 2).

Eleven groups of nymphs transmitted during the 21-25 hour feeding period, 6 during the 25-29 hour feeding period, 4 more during the 29-34 hour interval, 2 during the 34-46 hour period, and 3 groups transmitted only at the 46-70 hour interval. In all, 27 of the 30 lots proved infective. From this it is immediately evident that not only was a shorter latent period indicated, since in 11 cases no latent period was

TABLE 2.—*Results*[a] *of trials to determine the latent period of pea-enation mosaic virus in 1st instar pea aphid nymphs (Ave. age = 8.5 hrs.) tested in groups of threes after receiving an access feeding of 22 hours*[b]

Aphid lot no.	Total test feeding time in hours plus 21 hours of access feeding time				
	25	29	34	46	70
1	—	—	—	—	+
2	—	—	—	—	+
3	—	—	—	—	+
4	—	—	—	+	+
5	—	—	—	+	+
6	—	—	—	+	+
7	—	—	+	—	+
8	—	—	+	—	—
9	—	—	+	—	+
10	—	+	—	+	+
11	—	+	—	—	+
12	—	+	+	+	+
13	—	+	+	+	+
14	—	—	+	+	+
15	—	+	+	—	+
16	—	+	—	+	—
17	+	+	+	+	+
18	+	+	—	+	—
19	+	—	+	+	+
20	+	+	+	—	+
21	+	+	—	—	D
22	+	+	+	+	+
23	+	+	—	—	+
24	+	+	+	+	+
25	+	+	+	+	+
26	+	+	—	—	—
27	+	—	+	—	+
Total	11	15	14	14	22

[a] Plus (+) sign indicates that plant was infected; minus (—) sign signifies that plant remained healthy; and D indicates the plant died.
[b] Test feeding times of 4, 4, 5, 12, and 24 hours were used.

shown, but the efficiency of transmission of the nymphs seemed much higher.

Since 11 plants in the first series of test plants became diseased, another trial was made in which a shorter access feeding period was used to determine a minimum latent period. Two groups of first instar nymphs (average age = 6 hrs.) were placed on the same 2 source plants used in the previous experiment. They were given an access feeding of 13 hours and moved in lots of threes to 30 plants, 15 lots for each source. These groups were transferred serially to 4 test plants for test feeding periods of 4, 4, 3, and 12 hours, respectively. (Table 3).

None of the plants at the 12–16 hour interval became infected whereas 3 became diseased during the 16–20 hour feeding period. Two more groups became infective during the 20–23 hour interval and 4 groups transmitted for the first time at the 23–35 hour level. Whereas in the first trial 27 of the 30 aphid groups proved infective, only 9 of the 30 lots were vectors in this trial. Although the results are not strictly comparable, there is a definite indication that 1) length of access feeding, and 2) ensuing changes in vector

TABLE 3.—*Results[a] of trials to determine the latent period of pea-enation mosaic virus in 1st instar pea aphid nymphs (Ave. age = 6.0 hrs.) tested in groups of threes after receiving an access feeding of 13 hours[b]*

Aphid lot no.	Total test feeding time in hours plus 12 hours of access feeding time			
	16	20	23	35
1	—	—	—	+
2	—	—	—	+
3	—	—	—	+
4	—	—	—	+
5	—	—	+	—
6	—	—	+	—
7	—	+	—	—
8	—	+	—	—
9	—	+	—	—
Total	0	3	2	4

[a] Plus sign (+) indicates plant was infected; minus sign (—) signifies plant remained healthy.
[b] Test feeding times were 4, 4, 3, and 12 hours.

efficiency are limiting factors in any attempt to demonstrate a minimum latent period.

It appears possible therefore that there may be no real difference in the length of the latent period between first instar nymphs and adults but rather a pseudo-difference which can be explained on the basis of the greater efficiency of the nymphs as vectors. The following experiment yielded information on this point.

EFFICIENCY OF TRANSMISSION BY NYMPHS AND ADULTS.—Differences between nymphs and adults in ability to acquire virus have been reported for tomato spotted wilt (2), curly top of sugar beet (6), pupation disease of oats (13), and filaree red-leaf (1) viruses. Although Osborn (7) worked with both nymphal and adult pea aphids, both forms were such efficient vectors when given only a few hours access feeding it is probable that any differences in ability to acquire virus were masked.

In the present work first instar nymphs (ave. age = 8 hrs.) and adult apterous aphids were given access feedings of 1, 2, 4, 8, 24, and 48 hours on a common source plant. Two replications using single insects per test plant and 15 plants per treatment were carried out. The aphids were given a test feeding period of 4 days in order to nullify any latent period effect. Results of the trials are in Table 4.

Whereas none of the adults acquired virus before an 8-hour feeding period had elapsed, 13 of the nymphs became infective during this time. Whereas only 3 of the 30 adults transmitted at the 24-hour level, 10 of the 30 nymphs were vectors. Similarly, 2 of 20 adults compared to 11 of the 30 nymphs tested proved to be infective at the 48-hour level.

One possible explanation for the results is that there is a difference in feeding rates between the 2 forms. This is not too difficult to hypothesize since the growing nymphs could very well have a considerably higher rate of metabolism than the adults and thus might feed at a much faster rate.

TABLE 4.—*Results[a] of trials to demonstrate a difference in the ability of 1st instar nymphs and pea aphid adults to acquire pea-enation mosaic virus[b]*

Instar at time of access feeding	Access feeding time in hours						
	1	2	4	8	24	48	Total
1st	1/30	1/30	4/30	7/30	10/30	11/30	34/180
adult	0/30	0/30	0/30	0/30	3/30	2/20	5/170

[a] In above ratios numerator indicates number of infections obtained; denominator number of plants inoculated.
[b] Access feeding periods of 1, 2, 4, 8, 24, and 48 hours and a test feeding period of 96 hours were used.

POST-ACQUISITION FEEDING STARVATION.—It was demonstrated by Watson (18) that transmission of beet yellows, a persistent aphid-borne virus, was affected by starving infective aphids between the acquisition and test feeding periods. This starvation resulted in a drop in vector efficiency. Similar studies of this phenomenon have been done in connection with 2 other viruses of the above group, beet yellow-net (16), and filaree red-leaf (1). However, in neither case was any correlation between the length of post-acquisition feeding starvation and efficiency of transmission shown. Information concerning this phenomenon in respect to pea-enation mosaic virus was obtained. The following procedure was used. Approximately 250 adult pea aphids were given an access feeding period of 72 hours on a diseased bur clover plant. These insects were then placed in glass vials where they were starved for periods of 2, 4, 8, 12, and 24 hours. Individuals were placed singly on healthy bur clover seedlings for 24 hours. Thirty aphids were tested at each of the times. Also 30 unstarved aphids were placed on test plants to serve as a check group.

Results of the experiment were as follows: 7 of 30 unstarved individuals were vectors, 5 of the 30 transmitted at the 2-hour level of post-starvation, and 6 of 30 insects were infective at each of the other periods tested. Evidently, starving infective insects for periods up to 24 hours had no influence on ability of the pea aphid to transmit the virus. Possibly the demonstrable effect of starvation shown by Watson with beet yellows was correlated with the short retention time of that virus in the vector. Beet yellows is reported by Watson (18) to be retained for not over 72 hours whereas pea-enation mosaic and beet yellow net have been shown to persist for 10 days or longer.

DISCUSSION.—The results obtained during the course of this work appear in general to conform with the previously established patterns of vector-virus relationships for aphid-borne persistent viruses (1, 12, 16, 17, 18). The establishment of the short inoculation threshold period further characterizes the pea-enation mosaic virus as a member of this group of viruses.

One point which merits further consideration is the apparent difference between nymphal and adult instars

in regard to length of the latent period. If, as has been suggested, this is an apparent phenomenon due to differences in vector efficiency, it would seem possible that the so-called "minimum latent period" values which were found may also be apparent phenomena, reflecting the conditions under which the experimentation was done and not necessarily indicating the absolute "transmission threshold period." The fact that for 11 of the 30 groups of nymphs tested no latent period could be shown clearly indicates the possibility of the "minimum latent period," occurring considerably below the 16–20 hour limit demonstrated in a subsequent experiment. Whether this "minimum latent period" could be extended back to the point of non-existence can neither be proven nor disproven with these data but, in the author's opinion, it would seem that this might be the case. Certainly it is apparent that as one tries to shift this time back the difficulties encountered are greatly multiplied because of the necessity of using relatively short acquisition and test feeding periods. Only by utilizing large numbers of insects could one circumvent such unfavorable conditions.

That a discernible latent period does exist for most vectors would seem to be clearly indicated. The steadily increasing number of transmissions which appear in all the tables cannot be attributed to anything except that more and more groups of aphids are becoming infective as time progresses.

The explanation for the wide variation in the time it takes a vector to become infective is as yet unknown. There is some indication that it may be connected with the amount of virus taken up during the acquisition feeding period (4). An interesting trend in this regard was noted in connection with the work on effect of access feeding on retention where apparently the most highly infective aphids had the shortest latent periods. Of 60 insects which were given 48 or 96 hour access feedings, 13 proved to be vectors. Of these, 10 began to transmit either on the first or second day after leaving the source plant and infected 99 of the 259 (34 per cent) plants on which they fed, whereas the other 3 vectors caused their initial transmissions from the third to the eighth day after leaving the source plant and infected 7 of 70 (10 per cent) test plants.

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MECHANICAL AND APHID TRANSMISSION OF THE FEATHERY MOTTLE VIRUS OF SWEET POTATO [1]

R. E. Webb and R. H. Larson

SUMMARY

Mechanical- and aphid-transmission studies of the feathery mottle virus were initiated to determine its possible relationship to a sweet potato virus occurring in Wisconsin and Louisiana. The feathery mottle virus proved mechanically transmissible to sweet potato varieties Porto Rico Unit 1, Texas Bunch, Georgia Bunch, Ranger, and L240 and aphid-transmissible to Porto Rico Unit 1, Texas Bunch, and Georgia Bunch. Also, the virus was found to be transmitted to new plants through the fleshy roots.

Cross-protection tests indicated a close relationship between the feathery mottle virus and the naturally occurring sweet potato virus found in Wisconsin and Louisiana.

A virus disease of sweet potato resembling that caused by the feathery mottle virus of Doolittle and Harter[2] was found in central Wisconsin in 1951 and in 2 areas of Louisiana in 1952. The causal virus was found to be mechanically transmissible to sweet potato plants. In contrast, Doolittle and Harter reported failure to obtain mechanical transmission of the feathery mottle virus, transmission being accomplished by the root-plug and approach-grafting techniques. These authors did report, however, an indication of mechanical transfer; whereas all of 20 cleft-grafted infected scions died within 24 hours, 2 of the grafted stocks did develop typical vein-feathering symptoms.

Comparison of the symptoms of the feathery mottle virus with those induced by the virus from Louisiana and Wisconsin suggests identity of the causal viruses. Proof of mechanical transmission of the feathery mottle virus would supply additional evidence of identity. Therefore, mechanical transmission studies of the feathery mottle virus were initiated.

Sweet potato plants infected with the feathery mottle virus were received from S. P. Doolittle, Beltsville, Md., and kept in insect-proof cages. These plants served as the source of inoculum for mechanical- and insect-transmission studies. Virus-infected plants from Wisconsin and Louisana were used as sources of inoculum in comparative studies.

Inoculum was obtained by grinding virus-infected leaves with mortar and pestle and straining the juice through cheesecloth. The inoculum, diluted 1:10 with distilled water, was applied to healthy plants with sterile coarse cotton gauze. Carborundum, 600-mesh, was used as an abrasive.

Sweet potato plants used in these studies were kept in the greenhouse at 75–80°F. To prevent spread of virus by insects, the plants were sprayed 3 times weekly with an insecticide until discarded.

TRANSMISSION OF FEATHERY MOTTLE VIRUS.—Five plants each of the sweet potato varieties Porto Rico Unit 1, Texas Bunch, and Georgia Bunch were inoculated. Small faintly chlorotic dots appeared on the inoculated leaves 14–19 days after inoculation. The chlorotic areas gradually enlarged until they became confluent with the small veins along which the spread was rapid, causing typical vein feathering (Fig. 1,A). As the inoculated leaves aged, vein feathering faded, leaf enlargement was slightly retarded, and the inoculated leaves became chlorotic and looked slightly saddle-shaped. Similar results were obtained in 2 additional series of tests.

Two serial transfers of expressed sap from the mechanically inoculated plants were made to healthy plants of the 3 varieties mentioned. Symptoms induced by the virus from this source were similar to those produced by inoculum from the original host plants.

Symptoms induced by cleft-grafting with scions from the original host or from mechanically inoculated plants and by mechanical inoculation appeared to be identical on each of the varieties tested. Slight differences were noted in severity of symptoms but were attributed to the initial physiological conditions of the host plants.

Sweet potatoes are propagated by young sprouts produced by fleshy roots. If the virus is translocated to the fleshy roots, the sprouts produced would probably become virus sources when transplanted to the field. To test this possibility, mechanically inoculated and non-infected plants of Porto Rico Unit 1, Ranger, and L240 were planted in 8-in. pots for root production. After 110 days the fleshy roots were harvested and stored at 80°F. to hasten sprouting. Roots from healthy and virus-infected plants were then planted in pots for sprout production. Thirty-nine to 45 days after potting, the sprouts of the 3 inoculated varieties developed chlorotic dots which gradually developed into vein feathering (Fig. 1,B). Inoculum from virus-infected leaves of the plants induced symptoms identical to those from the original source.

Aphid transmission of the feathery mottle virus was attempted by placing non-viruliferous *Myzus persicae* (Sulz.) on virus-infected plants for a 3-day acquisition period. Twenty viruliferous aphids were then transferred by means of a camel's hair brush to 10 plants of each of the previously mentioned varieties. The aphids were allowed to feed 3 days and then were killed. Inoculated plants remained in insect-proof cages for 40 days. Of the 10 plants of each variety inoculated, the following numbers developed symp-

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The illustrations were prepared by Eugene H. Herrling, University of Wisconsin.

[2] Doolittle, S. P., and L. L. Harter, 1945. A graft-transmissible virus of sweet potato. Phytopathology 35: 695-704.

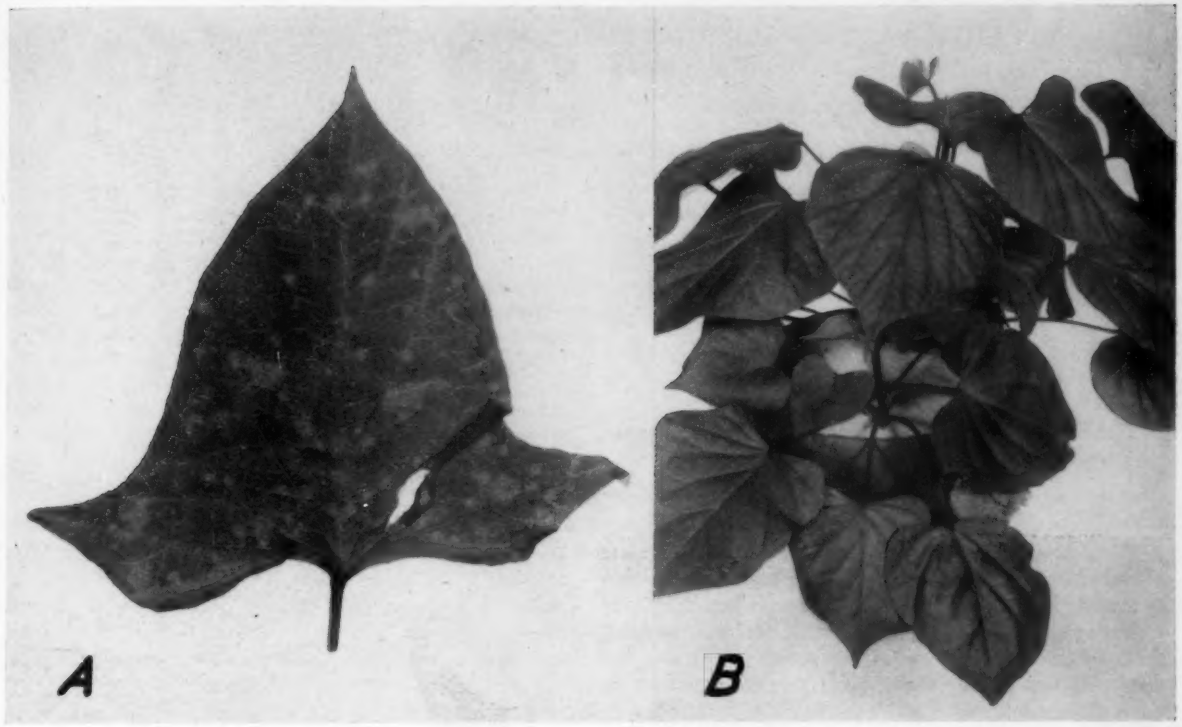

FIG. 1. A) Sweet potato leaf of variety of Porto Rico Unit 1 showing typical mottle. B) Slips produced by virus infected roots of the same variety showing mottle.

toms: Porto Rico Unit 1, 6 plants; Georgia Bunch, 4 plants; and Texas Bunch, 7 plants. A few typical yellowish dots and occasional vein feathering developed on all varieties 19–28 days after inoculation. These symptoms initially were not so numerous nor so severe as those induced by mechanical inoculation. Usually the leaf symptoms resulting from insect transmission were a chlorotic caste and slight dwarfing.

Systemic symptoms of virus-infected sweet potato plants were essentially the same whether inoculated by mechanical means or by aphids. Initially, the characteristic pin-point chlorotic spots appeared on the young leaves when they were ½–⅔ grown. Vein feathering developed subsequently, and as the infected leaves aged, they became slightly chlorotic. No obvious stunting was noted of infected plants of the varieties inoculated while growing in pots or when later transferred to the field. Actively growing infected plants usually showed all symptoms of the disease on the same plant. The mature leaves were slightly dwarfed and chlorotic, maturing leaves showed different degrees of feathering and slight chlorosis, whereas the uppermost leaves, ½–⅔ grown, showed chlorotic dots of various sizes and some feathering.

CROSS-PROTECTION TESTS.—The sweet-potato virus found in Wisconsin and later in Louisiana induced symptoms very similar if not identical to those caused by the feathery mottle virus on certain sweet potato varieties. Cross-protection tests were conducted to determine the relationship between the 2 viruses. Fifteen young plants of Porto Rico Unit 1, Texas Bunch, and Georgia Bunch were mechanically inoculated with each of the 2 viruses. Twenty-two days after the initial inoculations, 10 plants of each variety previously inoculated with the feathery mottle virus were inoculated with the unidentified virus. Similarily, 10 plants previously inoculated with the unidentified virus were inoculated with the feathery mottle virus. Also, 5 additional plants of each variety were inoculated with each of the 2 viruses.

Symptoms produced by the initial inoculations of the 2 viruses appeared about the same time on all varieties and were apparently identical in type, severity, and subsequent development. Symptoms that developed on the plants inoculated the second time with the different viruses were not altered in any manner. Symptoms on the control plants made at the second inoculation were very similar to those induced by the first group of inoculations. This confirmed the suspected relationship of the 2 viruses. A second series gave similar results.

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RELATIVE PATHOGENICITY OF FUNGI ASSOCIATED WITH ROOT ROTS OF RED CLOVER IN WISCONSIN [1]

R. A. Kilpatrick, E. W. Hanson, and J. G. Dickson [2]

SUMMARY

The principal conclusions from this study were that many of the species of fungi associated with diseased roots of red clover are able to infect this crop in the seedling stage and that there are wide differences in pathogenicity between isolates of most of the species concerned. Disease symptoms included discoloration and decay of the roots and hypocotyls, stunting, and sometimes death of the plants. Isolates of *Pythium*, *Fusarium*, *Rhizoctonia*, *Phoma*, *Gliocladium*, and the blackpatch fungus were among the most pathogenic.

Two methods of testing pathogenicity were used. The laboratory method, in which the tests were made in test tubes, was useful for quick preliminary tests. It was especially useful for eliminating non-pathogenic isolates, and required minimum equipment and space. It did not measure pre-emergence effects. The greenhouse method, in which the tests were made in sand culture in glass tumblers, also gave a quick test and had the advantage of measuring pre-emergence as well as post-emergence effects. Both methods gave consistent results in repeated tests.

Many fungi have been isolated from diseased roots of red clover (5). Some have been shown to be pathogenic (1, 2, 3, 4, 6, 7, 9) on this crop but relatively little is known concerning the relationships of most of them to the root and crown rot problem. It was the object of this study to obtain further information on the pathogenicity of fungi commonly associated with diseased roots of red clover in Wisconsin. Since all tests were made with pure cultures under strictly artificial conditions, it was not expected that the results would necessarily indicate what happens in the field. Rather, it was hoped that the information acquired might be useful in planning further studies of the problem. This paper is concerned only with the effects of the fungi studied on seedlings. Another paper will report their effects on older plants.

MATERIALS AND METHODS.—Several methods for determining relative pathogenicity were tried. The method found most satisfactory for laboratory tests was a modification of the technique described by Neergaard (8). It consisted of growing seedlings in combination with the fungi to be tested for 10 days under aseptic conditions on filter paper platforms suspended in sterile distilled water in test tubes (Fig. 1). The platforms were prepared as follows: Filter paper disks, 6 in. in diameter, were cut into quarters. Each quarter was shaped around a cylindrical piece of wood, slightly smaller than the test tube, into a cylinder with one end closed. This cylinder was then inserted into the tube with the closed end up and above the level of the water. Each tube, 15 mm. in diameter, contained 10 cc. of water. Five sterile seedlings, 48–60 hours old, were distributed on the top of each platform and an agar disc, 5 mm. in diameter, bearing the inoculum was placed between the seedlings. Fifty seedlings, 10 replicates of 5 seedlings each, were inoculated with each isolate. A full series of checks, seedlings grown in presence of sterile agar discs, were included in each trial. Isolates were completely randomized within each replicate.

Inoculum was increased on potato-cerelose-agar in Petri plates and was 5 days old when used. The seedlings were produced from a single seed lot of Midland red clover which was practically free from seed infection. The seed was surface sterilized prior to germination by dipping it in 50 per cent alcohol for 30 seconds, transferring it to a 0.1 per cent solution of mercury bichloride for 4–6 minutes, and then transferring it to a 1 per cent solution of calcium hypochlorite for 14–16 minutes. Immediately following this treatment, the seed was rinsed twice with sterile distilled water and placed on moist filter paper in Petri plates for germination. The plates were kept at room temperature for 48–60 hours when the seedlings were large enough to be transferred to the test tubes for inoculation.

During these tests the tubes were mounted on a large rack (8) to provide uniform light conditions and to facilitate observations which were made daily to note progress of disease development. Temperatures varied from 65° to 75°F. Ten days after inoculation, all seedlings were classified into 7 disease severity classes, as previously described (5), and a disease index calculated for each isolate. Check seedlings were still vigorous and in excellent condition at the end of the test.

The glass tumbler method described by Halpin et al (4) was used for the greenhouse tests. One hundred red clover seeds were sown per tumbler in a substratum of white silica sand, 1 normal Hoagland's solution, and a small amount of agar bearing the fungus to be tested. The seed used was from a lot of Wisconsin Mildew Resistant produced at Prosser, Washington and known to be virtually free of seed infection. Its germination on blotters was 85 per cent and it was surface sterilized prior to use in the same

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[2] Appreciation is expressed to E. H. Herrling for assistance in the preparation of illustrations.

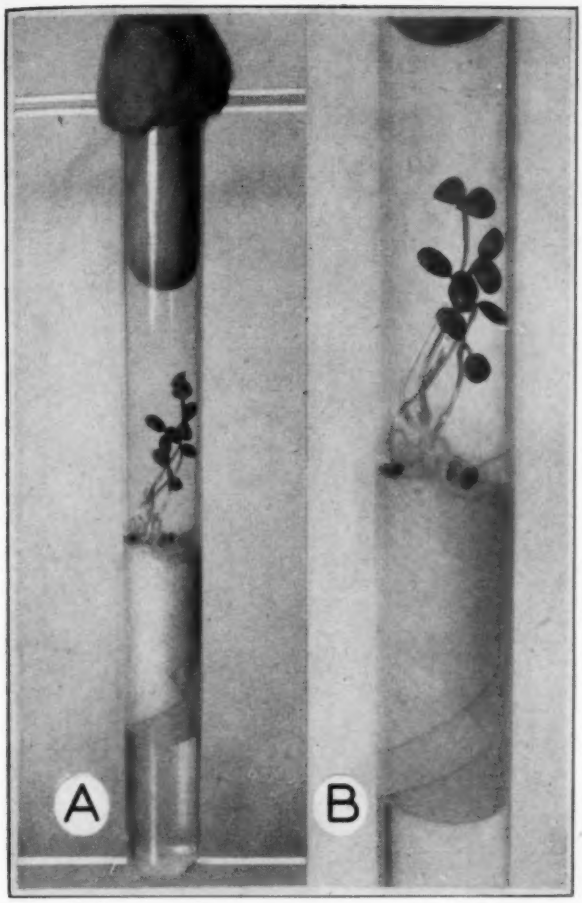

FIG. 1. Equipment used in laboratory pathogenicity tests. A) Test tube with filter paper platform in place and extending into water. B) Platform and seedlings enlarged.

manner as the seed used in the laboratory tests. It would have been better to have used a single seed lot for both the laboratory and greenhouse studies but the supply of seed used in the former was not adequate for both tests. Immediately after seeding, the tumblers were placed in a greenhouse with a constant temperature of 28°C. This temperature was selected after preliminary studies had indicated that the majority of the isolates produced greater mycelial growth and a higher level of disease at this temperature than at lower temperatures. Kreitlow and Hanson (6) have reported maximum disease development with *Fusarium* spp. at temperatures above 30°C. but the writers have found that temperatures of that magnitude frequently have an adverse effect on red clover seedlings. Four replicates of each isolate were included in each test. Each replicate was a completely randomized block and all tests were repeated. Daily observations were made to note progress of disease development and severity ratings were determind 14 days after inoculation.

All the fungi tested for pathogenicity in these investigations were isolated in 1949 and 1950 from red clover roots grown in Wisconsin.

RESULTS. — *Laboratory tests.* — The results of the pathogenicity tests made in the laboratory with 312 of the isolates tested are summarized in Table 1. They show: 1) that many of the fungi studied could infect red clover seedlings, and 2) that there were wide differences in pathogenicity between isolates of most of the species. Isolates of *Fusarium oxysporum* ranged in severity from 26 to 95, those of *F. solani* from 34 to 95, and those of *F. roseum* from 23 to 95. Isolates of *Rhizoctonia* varied from 8 to 95. Isolates of *Chaetomium*, *Mucor*, *Aspergillus*, *Alternaria*, *Penicillium*, and *Trichoderma* were in general either nonpathogenic or only weakly pathogenic. All these genera except *Chaetomium*[3] included isolates that incited some lesions and discoloration on the roots.

Greenhouse tests.—Results of the tests made in the greenhouse are summarized in Table 2. They show that all the isolates of *Pythium debaryanum* tested and some of the isolates of *Fusarium oxysporum*, *F. roseum*, and *F. solani* were very pathogenic on red clover seedlings. Only 1 isolate of the blackpatch fungus[4] (7) was tested but it also was very pathogenic. Some isolates of *Rhizoctonia*, *Phoma*, and *Gliocladium* were moderately pathogenic. In most species there were some isolates that proved either non-pathogenic or only very weakly pathogenic. In general, there was a wide range in the pathogenicity of isolates of the same species. As shown in Table 2, there was good agreement between the results obtained in the greenhouse and laboratory experiments. On the average, and with

[3] Only 1 isolate tested.

[4] Isolated from roots of red clover in Wisconsin in 1950; identification checked by J. G. Leach.

TABLE 1.—*Relative pathogenicity of 312 fungus isolates on red clover seedlings in laboratory tests*

Fungus	Number of isolates tested	Disease severity rating[a]	
		Range between isolates	Average
Curvularia sp.	1	79	79
Fusarium solani	55	34—95	75
Gliocladium roseum	10	50—95	75
Fusarium oxysporum	72	26—95	74
Fusarium roseum	34	23—95	74
Rhizoctonia spp.	34	8—95	64
Helminthosporium sp.	3	39—73	59
Pythium spp.	8	22—82	56
Colletotrichum destructivum	1	46	46
Phoma spp.	15	6—87	41
Penicillium spp.	7	1—50	35
Trichoderma spp.	10	15—63	33
Fusarium moniliforme	1	25	25
Alternaria spp.	8	1—68	24
Aspergillus spp.	3	7—34	21
Mucor spp.	18	1—66	18
Chaetomium sp.	1	0	0
Miscellaneous	31	0—94	37

[a] 0 = no disease; 100 = all plants killed.

TABLE 2.—*Relative pathogenicity of 28 fungus isolates on red clover seedlings as indicated by disease severity ratings and effects on stand*

Fungus and isolate No.	Disease severity rating[a]		Stand in greenhouse tests	
	Laboratory tests	Greenhouse tests	Per cent plants emerged[b]	Per cent stand 14 days after seeding[b]
Check	0	0	71[c]	71
Fusarium oxysporum				
5	26	8	70	67
258	94	58	57	46
29	95	83	67	11
251	57	95	51	1
Fusarium solani				
51	86	7	71	68
106	69	20	68	61
300	90	74	62	19
22	54	80	71	13
Fusarium roseum				
92	60	11	62	60
229	95	50	65	47
76	58	59	68	39
254	95	92	51	3
Phoma spp.				
146	6	1	71	71
212	22	15	58	51
221	74	62	53	37
257	81	74	56	31
Rhizoctonia spp.				
136	16	4	73	73
74	94	19	61	54
250	89	63	66	36
45	59	50	60	36
Gliocladium roseum				
6	50	64	76	76
176	84	67	70	68
228	84	68	69	47
Pythium debaryanum				
164	68	91	10	2
67	66	95	14	0
208	80	95	2	0
Pythium sp.				
131	47	86	51	12
Blackpatch fungus				
134	94	95	62	2

[a] 0 = no disease; 100 = all plants killed.
[b] Average stands for all tests.
[c] The seed lot used germinated 85 per cent on blotters but only 71 per cent of the seeds sown produced plants that emerged in these tests. Check seedlings were free from disease.

the exception of the isolates which caused considerable pre-emergence killing, the laboratory method gave a somewhat higher level of disease. It was expected that this method would give lower disease severity ratings in cases where pre-emergence killing was involved since it was not designed to measure this type of injury. The results obtained with the *Pythium* isolates and some of the more pathogenic of the other isolates illustrate this point.

Stand losses ranged from 0 to 100 per cent (Table 2). *Pythium debaryanum* isolates 67, 164, and 208, the blackpatch fungus 134, *Fusarium oxysporum* 251, and *F. roseum* 254 caused almost complete killing of seedlings in 14 days. In contrast, *Rhizoctonia* 136, *Phoma* 146, and *Gliocladium* 6 had no effect on stand. Most isolates of *Gliocladium*, however, caused severe stunting of the seedlings.

Symptoms incited by pathogenic isolates included pre-emergence killing, post-emergence damping-off, stunting, and rotting of the roots and hypocotyls. Fig. 2 shows the effects of isolates 208, 251, 300, 257, 134, 250, 6, and 229, respectively, on the above ground parts of the seedlings. The most severe type of pre-emergence killing was caused by *Pythium debaryanum* (Fig. 2, B). Many pathogenic isolates caused some post-emergence damping-off. The amount varied greatly among isolates. This type of symptom is illustrated in Fig. 2, C, E, and F, where *Fusarium oxysporum* 251, *Phoma sp.* 257, and isolate 134 of the blackpatch fungus, respectively, were used. Some isolates caused wilting (Fig. 2, C); others caused mostly stunting (Fig. 2, H). Most of the pathogens caused a reduction in the size of the seedlings but it was not always the smallest seedlings that damped-off most

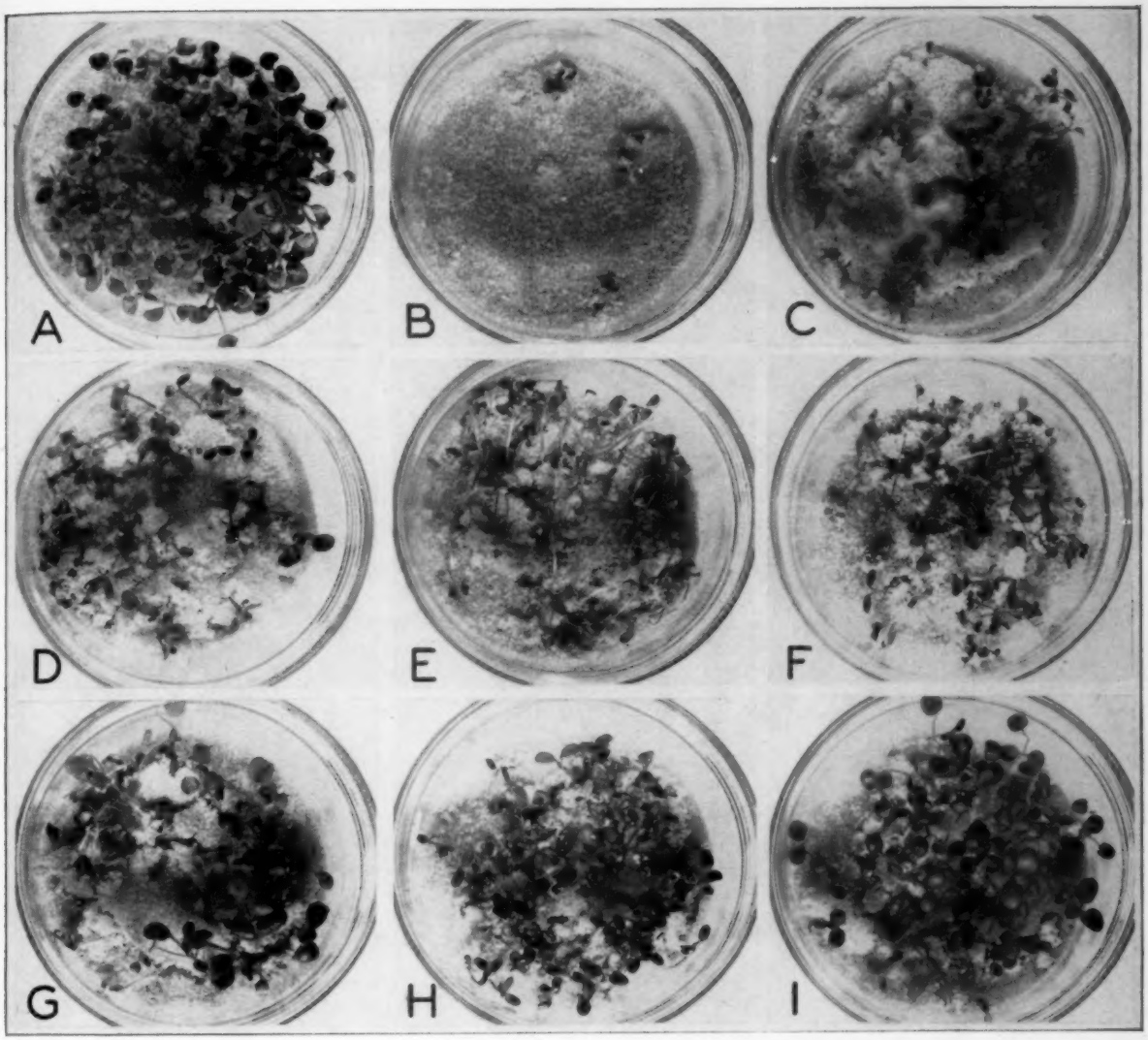

FIG. 2. Top view of glass tumblers showing the effects of 8 fungus isolates on red clover seedlings 13 days after seeding in sand culture at 28°C. A) Check; B) *Pythium debaryanum;* C) *Fusarium oxysporum;* D) *F. solani;* E) *Phoma sp.;* F) the blackpatch fungus; G) *Rhizoctonia sp.;* H) *Gliocladium roseum;* I) *Fusarium roseum.*

rapidly. *Gliocladium roseum* caused severe stunting but did not kill many plants during the 2-week period following inoculation.

Fig. 3 shows the effects of the same isolates on the roots and hypocotyls of seedlings 15 days after inoculation. *Pythium debaryanum* (Fig. 3, E) infected and killed the plants in an early stage of development. It caused some seed decay but in most cases the seeds germinated and the seedlings were subsequently killed prior to emergence. All parts of the seedling were attacked. The most pathogenic isolates of *Fusarium oxysporum* (Fig. 3, B) caused a severe necrosis of the upper part of the primary root and lower part of the hypocotyl. Sometimes the decay extended well up on the hypocotyl. Wilting was common. Some isolates of this fungus produced considerable aerial mycelium on the surface of the sand and on the above ground parts of the plants (Fig. 2, C), however, this did not happen until the seedlings were already wilted and dying. *Fusarium solani* caused a severe dark brown to black decay of the entire root system (Fig. 3, D). Both *Phoma* and *Rhizoctonia* isolates attacked the seedlings at the soil line causing a dark brown to black necrosis of the upper part of the primary roots and lower part of the hypocotyls (Fig. 3, C, G). When the rotting was severe the seedlings would fall to the

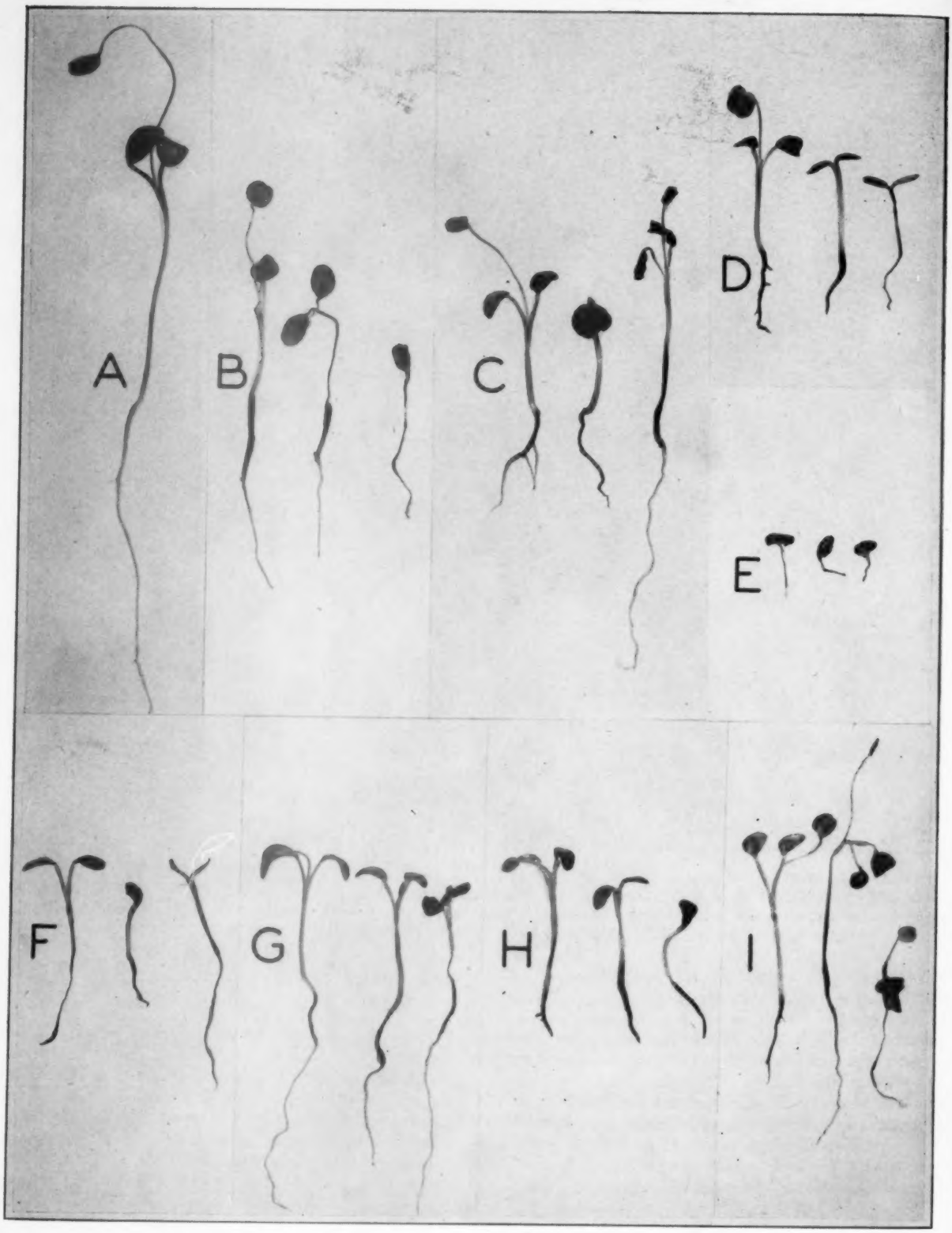

FIG. 3. Red clover seedlings showing symptoms incited by 8 fungus isolates. A) Check, not inoculated; B) *Fusarium oxysporum*; C) *Phoma sp.*; D) *Fusarium solani*; E) *Pythium debaryanum*; F) Blackpatch fungus; G) *Rhizoctonia sp.*; H) *Gliocladium roseum*; I) *Fusarium roseum*.

ground and die. The blackpatch fungus attacked the entire root system and commonly also the aboveground parts of the plants (Fig. 3, F). *Gliocladium roseum* caused a dark brown rot of the roots and frequently of the lower part of the hypocotyl (Fig. 3, H). The pathogenic isolates of *Fusarium roseum* caused a brown rot of the roots and lower part of the hypocotyls (Fig. 3, I).

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THE INFECTION OF NICOTIANA GLUTINOSA WITH TOBACCO MOSAIC VIRUS AS AFFECTED BY POTASSIUM NUTRITION [1]

William B. Allington and Edward F. Laird, Jr. [2]

SUMMARY

Low potassium nutrition increased the tendency for *Nicotiana glutinosa* plants to become infected with tobacco mosaic virus. In some experiments the amount of infection in the low potassium plants was greater when inoculated up to 1 minute after wounding. Generally, the virus receptive condition persisted longer and at a much higher level in the low potassium plants.

The mechanism of virus infection in plants is obscure at the present time and the problem has not been found amenable to direct methods of investigation as with bacterial viruses. Studies such as those involving the action of inhibitors have greatly stimulated thought on the problem but no direct evidence of the nature of the processes involved has been presented. A concept which has gained general acceptance is that virus particles are mechanically introduced into plant cells at the instant injury takes place and that the cells that initially become infected do so because they recover sufficiently from the mechanical injury so that some semblance of their normal functions is retained. This perhaps has been a logical hypothesis. However, with increasing evidence as to the nature of the infection phenomenon with bacterial viruses and as more is learned about plant and animal virus infection, this hypothesis does not appear to be adequate.

Sheffield (4) and Kalmus and Kassanis (2) have presented evidence which indicated the tendency for wounded or rubbed leaves to remain susceptible or receptive to tobacco mosaic virus for various periods of time. The greatest amount of infection took place immediately after wounding and the tendency decreased steadily, but some infection still was possible without further wounding for as long as several minutes.

The results reported in this paper are concerned with the effect of potassium nutrition on the duration of the receptive condition of wounded leaf areas to tobacco mosaic virus establishment. Spencer (5) and Bawden and Kassanis (1) have studied the effect of nutrition on the number of infection points produced with tobacco mosaic virus. Spencer found that when plants were severely starved for potassium, small amounts of this element increased susceptibility but further increasing amounts decreased susceptibility. On the other hand, Bawden and Kassanis conclude that the main influence of nutrition on the tendency of

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[2] Plant Pathologist and formerly Assistant in Plant Pathology respectively.

plants to become infected with tobacco mosaic virus is a result of the effect on the growth response of the plant; there are effects on the susceptibility to infection per unit area of leaf but these are small relative to the effect of plant size.

MATERIALS AND METHODS.—*Nicotiana glutinosa L.* plants were used to measure quantitatively the tendency of the plants to become infected with tobacco mosaic virus under different conditions. The number of local lesions produced was used as a criterion of the receptivity of the tissue to the establishment of the virus infection. The plants were started in soil as seedlings and at an early stage (3-5 true leaves) were transplanted into white quartz sand in glazed crocks to which complete nutrient solution was added at regular intervals. When the plants were approximately 10 in. in height, the crocks were flushed thoroughly with distilled water and then a nutrient solution lacking potassium was added to half the crocks; the remainder continued to receive the complete nutrient solution. The plants were used at the time of first appearance of signs of potassium deficiency. Very little difference in size of plants existed within each experiment at the time of the inoculation tests. The compositions of the nutrient solutions are presented in Table 1.

Tobacco mosaic virus for inoculum was partially purified from infective tobacco extract by alternating differential centrifugations at about 4,000 and 55,000 times gravity. The desired concentrations were made by appropriate dilutions of the stock concentrate at the time of the experiment. Uniform inoculum was always used on all leaves of both high and low potassium plants in each experiment and all inoculations were made within a period of 1 hour. All leaves were dusted with 600 mesh carborundum before rubbing. Virus was applied to the leaves at various intervals after rubbing. The leaves were, therefore, rubbed with fingers with no free moisture present and at the desired time the leaves were removed from the plant at the base of the petiole and plunged into the inoculum. This was followed by rinsing in clean tap water under conditions which could permit no further wounding. The detached leaves were incubated with their petioles in pans of water at 65°F. under fluorescent lights and under relative humidity conditions approaching 90 per cent. Local lesion development proceeded well under these conditions and lesion counts were taken 3-4 days after inoculation.

TABLE 1. *Composition of nutrient solutions used for growing N. glutinosa plants in sand cultures for virus infection studies*

Chemical	Grams per liter of nutrient solution	
	Low potassium	High potassium
$Ca(NO_3)_2 \cdot 4H_2O$	1.180	1.180
KNO_3	——	0.505
$NaNO_3$	0.425	——
NaCl	0.128	0.128
$MgSO_4 \cdot 7H_2O$	0.492	0.492
KH_2PO_4	——	0.136
$NaH_2PO_4 \cdot H_2O$	0.138	——
H_3BO_3	tr	tr
$MnCl_2 \cdot 4H_2O$	tr	tr
$FeSO_4$	0.005	0.005

The treatments in regard to time of inoculation were arranged in a Latin square design. Usually 5 treatments were made using 5 plants and 5 adjacent leaf positions per plant. The leaves selected for inoculation were those nearest the top which were judged to be fully expanded.

RESULTS.—Experiments on high and low potassium plants were made using a wide range of time intervals. The design of these experiments varied so greatly in this respect that all the tests are not conveniently amenable to general summarization. Results of 4 tests were selected as representative and are summarized in Table 2. Trends established in this table are typical of those in over 20 additional experiments using different time intervals.

Low potassium plants were typically more heavily infected than the high potassium plants. In addition they remained receptive for a longer period of time. Of special interest are the results of Experiment 1 where a statistically significant increase in infection resulted when inoculation was delayed as much as 1 minute after wounding. This observation has been made on low potassium plants in many other experiments not reported here. It becomes particularly significant when compared to the parallel high potassium series. A similar trend, although not significant, can be noted in Experiments 2 and 4. Considerable variability was experienced in many experiments but rarely were results obtained which were not strongly in support of the previous statements.

Because of limitations imposed on the experiments in order to conform to a statistical design, it was not convenient in the experiments reported to include enough treatments to establish a time-end point of infection for the low potassium series. Such a design would have decreased the number of treatments involving addition of the virus at shorter intervals and results such as found in Experiment 1, where an actual increase in infection resulted, would probably not have been obtained. A definite decrease in infection was typical, however, after four minutes in low potassium plants, but in some experiments the infection was still at a high level after ten minutes. Since the times selected for the experiments were determined primarily by the appearance of potassium deficiency symptoms, much of the variation from one experiment to another, therefore, was probably due to variability in the plants.

DISCUSSION.—The results reported here support the earlier finding of Spencer (5) that greater susceptibility to tobacco mosaic virus occurs when small amounts of potassium are added to low potassium plants. The term susceptibility as used here refers only to the tendency for the successful establishment

TABLE 2. *The effect of potassium nutrition on the duration of receptivity to tobacco mosaic virus in wounded N. glutinosa leaves*[a]

Time interval in seconds between wounding and inoculation	Average number of local lesions per leaf							
	Exp. No. 1		Exp. No. 2		Exp. No. 3		Exp. No. 4	
	Low K	High K	Low K	High K	Low K	High K	Low K	High K
0[b]	95	82	501	207	133	141	538	211
15	117	39	499	168	153	144	663	172
30	194	33	457	110	119	122		
45	338	25	642	66	153	99		
60	180	23	541	69	127	91	524	73
240							471	42
LSD 5%	66.3			64.7				65.0
LSD 1%		27.4						

[a] No statistical significance was found except in the experiments where an LSD is indicated.

[b] Leaves were rubbed with no virus present and plunged into inoculum with a minimum of time delay. Zero time therefore is not entirely accurate but the short amount of elapsed time was not measurable under the conditions of the experiment.

of a relationship between the host and virus which permits subsequent virus multiplication. It is admitted that the method employed, which is based on the development of local lesions, also utilizes the multiplication of the virus in the host. It is doubtful, however, that the length of the waiting period between wounding and exposure to virus would alter materially the conditions affecting multiplication after initial establishment.

The maintenance of a more receptive condition to virus after wounding in the low potassium series is of special significance. The actual increase in the amount of infection after a waiting period emphasizes the dynamic nature of this receptive condition. Results obtained with the complete nutrient solution series are typical of those reported earlier by Sheffield (4) and Kalmus and Kassanis (2).

Various possibilities exist which might explain the results reported here with low potassium. Since sodium ions were substituted for potassium ions the remote possibility exists that the effects attributed to low potassium may be associated with increased sodium. It is difficult to offer a hypothesis, however, which is consistent with the view that successful establishment of virus depends on the direct introduction of infective units into the slightly wounded cells. The increased infection rate observed up to a minute after wounding in the low potassium plants does not favor this viewpoint.

Relatively little is known concerning the functions of potassium in plants. Its high mobility and apparent "regulatory" properties on cell membranes are generally recognized. The prospects seem good that these properties are of importance in virus infection as studied here. Possibly the cells which are infected after wounding of a tissue are not necessarily those which are wounded, but others in close proximity, which have been temporarily affected and in which such ions as potassium, might exist in imbalance, thus resulting in the virus receptive period. The more plentiful the potassium the shorter this period might be. In the case of high potassium plants, present methods might be inadequate to detect any rapid initial rise of receptivity. Such views would bring the concept of the mechanism of plant virus infection into accord with those now being formulated for bacterial and animal viruses (3).

The relation of low potassium to infection with plant viruses other than tobacco mosaic virus should be investigated. Possibly studies of this type might help clarify present problems relative to insect specificity and other infection phenomena.

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FACTORS IN THE DEVELOPMENT OF BLUE DAMAGE OF SPANISH PEANUTS [1]

Don C. Norton

SUMMARY

Medium filtrates of various fungi, selected soils, and chemicals of known purity were tested in production of blue damage of peanuts. Of the fungi tested, only medium filtrates of *Sclerotium rolfsii*, *Aspergillus niger*, and *A. flavus* produced blue damage. A culture of *S. rolfsii*, in which oxalic acid was removed, did not produce blue damage.

Oxalic acid, various salts of oxalic acid, kojic acid, and several inorganic salts caused blue damage when mixed with shelled nuts and leached white quartz sand between pH 2.2–pH 7.0. Oxalates caused blue damage over a wider pH range than inorganic salts.

Blue discoloration also resulted when nuts contained in broken shells were mixed with acid soils with an approximate pH 6 or below. No discoloration occurred with similar experiments employing alkaline soils. No blue damage resulted when nuts were mixed with moist leached white quartz sand whose pH was lowered to a range between pH 6.0 and pH 2.0 by addition of HCl. It is probable that the ability of certain soils to produce blue damage is linked with an acid pH and with the presence of various ions in those soils.

The same agents and conditions under which blue damage is produced in Spanish peanuts were found to cause the discoloration in Virginia Bunch and Southeastern Runner type nuts but to a lesser degree.

Occurrence of blue damage of Spanish peanuts is usually associated with high humidity around the fruits during curing and storage. This damage has been described adequately by Garren et al[2] as "blue-black discoloration". The authors attributed the symptoms to an indicator reaction involving pigments of the seed coat and oxalic acid produced by associated fungi. *Sclerotium rolfsii* Sacc. was, in their experiments, the principal fungus involved. Although the authors recognized the possibility that other organic acid producing fungi may cause a blue-black discoloration, no further observations were made.

In Texas, in late summer and early autumn of 1952, blue damage occurred both in the field and laboratory in the complete absence of *S. rolfsii*. Among the more common fungi noticed as mycelial mats on shells were *Aspergillus flavus* Lk., *A. niger* V. Tiegh., and a species of *Penicillium*. Many other soil-borne fungi isolated from peanut shells were less frequent than the aforementioned fungi, and growth on the shell was much less vigorous. The frequency of isolation of *S. rolfsii* from the fruits varied, depending upon the location of the field from which the peanut sample was taken.

During preliminary tests, *S. rolfsii*, *A. flavus*, *A. niger*, *Rhizopus nigricans* Ehr. and 2 species of *Penicillium* caused blue damage when mixed with peanuts and moist Windthorst fine sandy loam soil. Addition of dextrose increased the damage. No discoloration occurred in the check when only nuts were mixed with moist soil.

The present work was undertaken to investigate the role of various chemicals in blue damage and the conditions under which the disease might develop.

MATERIALS AND METHODS.—Fungus cultures used in the preliminary experiments were maintained for 35 days on a yeast extract–5 percent dextrose solution. At the end of the period, 5 ml. of the autoclaved filtrate were added to 95 gm. of sterilized thoroughly leached white quartz sand in 250 ml. Erlenmeyer flasks. Shelled nuts were added, mixed well, and kept for 24 hours at 30°C. Oxalic acid was removed from a sample of the filtrate from the *S. rolfsii* culture by addition of a solution of Ca Cl_2 (followed by filtering) until no more precipitate of calcium oxalate formed. Five ml. of this filtrate were mixed with sand by the usual procedure. Nuts were soaked in filtrates of the cultures for 24 hours. The culture medium and water alone, both with and without sand, were used as checks. To study agents which may possibly cause blue damage, various chemicals were mixed with nuts and moist leached white quartz sand and kept in the dark for 24 hours at 30°C. In many instances, the pH was reduced by addition of small amounts of HCL.

Almost all experiments were repeated 1 or more times with uniform results; the material presented is representative. The disease index rating, ranging from 0.0 (no discoloration) to 10 (100 per cent of the testa showing intense blue damage) represents the area of the peanut affected and the intensity of blue discoloration of all nuts of a treatment. Generally, the amount of damage was distinct; either all peanuts in a given treatment had blue damage or none had.

The fungi selected were those most commonly iso-

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The writer expresses his gratitude to Dr. B. B. Higgins of the Georgia Agricultural Experiment Station, and to Dr. H. S. Ward, Jr. of the Alabama Polytechnic Institute for supplying the Virginia Bunch and Southeastern Runner type nuts used in this investigation.

[2] Garren, K. H., B. B. Higgins, and J. G. Futral. 1947. Blue-black discoloration of Spanish peanuts. Phytopathology 37: 669-679.

TABLE 1.—*Discoloration of peanuts as caused by filtrates of fungi*

Fungus	Nuts soaked in filtrate: Disease index	Nuts mixed with sand containing filtrate: Disease index	pH	Type of discoloration
R. nigricans	0.0	1.7	6.9	greenish brown
A. flavus	0.0	2.1	7.2	brown spotting plus moderate blue damage
A. niger	0.0	7.4	3.9	intense blue damage
Pencillium sp. #1	0.0	4.0	7.3	brown spotting
Penicillium sp. #2	0.0	2.3	7.0	brown spotting
S. rolfsii	0.0	5.1	5.8	intense blue damage
S. rolfsii minus oxalic acid	0.0	0.7	3.8	faint bleaching
Medium check	0.0	0.8	6.6	faint bleaching
Water check	0.0	0.0	7.3	none

lated from shells or nuts. The organic acids tested were chosen largely because of their reported production by 1 or more of the fungi used.

RESULTS.—Culture filtrates.—Only the filtrates of *A. niger* and *S. rolfsii* produced intense blue damage in nuts mixed with sand whereas *A. flavus* produced moderate blue damage (Table 1). No blue damage occurred from culture filtrates of other fungi or in the checks. When oxalic acid was removed from culture filtrates of *S. rolfsii*, only a faint bleaching resulted. *S. rolfsii* and *A. niger* were the only fungi to give a positive oxalate test using standard techniques. *A. flavus* was the only one to produce a positive reaction for kojic acid using the ferric chloride test.

Chemicals.—Kojic acid (Fig. 1), oxalic acid (Fig. 1) and many oxalates were capable of causing blue damage but its occurrence was at least partially dependent upon pH (Table 2). To determine whether or not cations are responsible for blue damage, various inorganic salts were mixed with peanuts in leached white quartz sand in the usual manner. Blue damage was readily obtained with KCl, NaCl (Fig. 1), and $CaCl_2$ when the pH was between 2.5 and about 6. When the pH was near the neutral point, no blue damage was observed, nor was it obtained with HCl alone, even when the resulting pH was between 2.0 and 6.0. Calcium nitrate and potassium nitrate produced a trace of blue damage at pH levels of 6.5 and 6.7, respectively. Except with the chlorides, the pH usually was not adjusted by adding HCl, and thus the pH of most of the phosphates, carbonates, and nitrates tested was near 7 or above, in which range there was no blue damage. The pH range, as given above, in which blue damage was caused by inorganic salts was not absolute and varied slightly with the chemicals. For example, ammonium chloride produced blue damage at a lower pH than did other chlorides.

Influence of cracked fruits.—During the process of harvesting and threshing peanuts, a large number of fruits became cracked and broken, and in some cases appreciable amounts of soil became mixed with the fruits. Twice as much blue damage occurred, on a percentage basis, from nuts taken from broken rather than unbroken shells. When shelled nuts were mixed with Lufkin fine sandy loam soil (pH 5.2), severe blue damage resulted, whereas nuts mixed with Windthorst fine sandy loam (pH 6.3) had no blue damage. Since the minerals in the soil might play a part in the cause of this discoloration, whole fruits, which had no breaks or shell discoloration, were split at the stylar end and broken open just wide enough to admit slightly damp soil when shaken in flasks. Similarly treated uncracked fruits were used as checks and kept for 48 hours at room temperature. The only appreciable blue damage developed when broken nuts were mixed with

TABLE 2.—*Disease index of blue damage resulting from chemicals mixed with quartz sand*

Chemical	Quantity gm. per 100 gm. sand	Disease index	pH[a]
Oxalic acid	0.1	3.0	2.3
Oxalic acid	1.0	0.0	1.3
Calcium oxalate	0.1	0.3	6.9
Calcium oxalate	1.0	5.4	3.7
Calcium oxalate	1.0	0.0	0.6
Sodium oxalate	0.1	5.6	7.4
Sodium oxalate	1.0	2.1	7.6
Potassium oxalate	0.1	4.4	7.4
Potassium oxalate	1.0	2.3	7.3
Potassium oxalate	1.0	0.2	1.8
Ammonium oxalate	0.1	5.5	7.0
Ammonium oxalate	1.0	2.2	6.5
Ammonium oxalate	1.0	5.5	4.8
Kojic acid	0.1	1.1	6.4
Kojic acid	1.0	1.6	5.6
Kojic acid	1.0	2.4	5.4
Succinic acid	0.1	0.0	3.3
Succinic acid	1.0	0.0	2.6
Citric acid	0.1	0.0	2.9
Citric acid	1.0	0.0	2.3

[a] Adjusted with HCl.

FIG. 1. Blue damage as produced by various chemicals. Left to right: check, oxalic acid, kojic acid, potassium chloride, and sodium chloride.

Lufkin soil (Table 3). In nearly all cases, discoloration was on the end of the nut adjacent to the break. In a similar manner, other acid soils were found capable of producing blue damage whereas alkaline soils were not.

Blue damage in Virginia Bunch and Southeastern Runner Type nuts.—The only report known to the writer where blue damage occurs on peanuts other than the Spanish type is that of Penny[3] who found 0.06 per cent blue discoloration in samples of the runner type nut.

Brief tests were made with Virginia Bunch and Southeastern Runner type nuts using methods employed previously. Although the intensity of damage was less, the agents and conditions which produced blue damage in Spanish peanuts also caused the discoloration in the bunch and runner type nuts.

[3] Penny, N. M. 1952. Economic aspects of peanut marketing. Ga. Agri. Exp. Sta. Bul. 273.

TABLE 3.—*Incidence of blue damage after 48 hours when cracked (stylar end) and uncracked fruits were mixed with 2 different soils*

Location of nut in shell	Windthorst fine sandy loam pH 6.3		Lufkin fine sandy loam pH 5.2	
	Uncracked	Cracked	Uncracked	Cracked
	No.	No.	No.	No.
Stem end	220	207	204	203
Blue damage	2	0	0	7
No blue damage	218	207	204	196
Stylar end	220	207	204	203
Blue damage	2	5	1	151
No blue damage	218	202	203	52

DISCUSSION.—The results of the work presented in this paper confirm that of Garren et al in that oxalic acid can cause blue damage in Spanish peanuts. Kojic acid, which is known to be produced by many species of *Aspergillus*, among them *A. flavus*, was also found capable of causing the discoloration. During current and related investigations, this fungus was, by far, the most common one isolated from shells and nuts. However, to what extent kojic acid is produced naturally in a peanut substrate is not known. Since other organic acids did not produce blue damage, except under abnormally high temperatures, it seems that oxalic and kojic are the organic acids most likely to cause damage in field and storage.

It is probable that much of the blue damage occurring in field and storage results from contamination of nuts by damp soil. In Texas, some soils in which peanuts are grown have a reaction of near pH 6. When the writer lowered the pH of the Windthorst soil with HCl and peanuts were mixed with it, blue damage developed. Since blue damage can result after contact of nut and soil for only a short time, rapid drying is advisable for this reason as well as to retard fungal growth. In Texas, the plants are usually left in the windrow for several days. If humid weather prevails, time is sufficient for blue damage development in nuts contained in broken shells.

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CUCUMBER AS A PLANT HOST IN STONE FRUIT VIRUS RESEARCH

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SUMMARY

A virus disease was readily incited in cucumber (*Cucumis sativus* L.) by mechanical inoculation with expressed juice from leaves of sour cherry (*Prunus cerasus* L.) and numerous other *Prunus* species infected with the necrotic ring spot virus alone or with the necrotic ring spot and yellows viruses. Symptoms on cucumber were found to be strikingly different from those incited by any virus previously known to infect cucumber. The cotyledons on plants inoculated with the virus from cherry usually persisted as yellow, mottled, turgid and functional organs for many weeks. The growing points usually were killed. Bud proliferation without elongation was common at the node or nodes below the killed growing point, with the subsequent development of a compacted rosette of many flowers and dwarfed leaves.

A high percentage of cucumber plants was infected and the disease development was maximum when a) the juice from young cherry leaves showing the initial symptoms of necrotic ring spot was used as the inoculum, b) vigorous young cucumber seedlings were inoculated on the cotyledons just as the primary leaf was beginning to unfold, c) the cucumbers were grown at pre- and post-inoculation temperatures of 24–28°C., and d) the cucumber plants were given supplemental light during the short days of winter. The virus was readily transferred mechanically from cucumber to cucumber with no apparent loss in virulence. It was not found possible to transmit the virus from cucumber to cucumber by the melon aphid, *Aphis gossypii* Glover, nor by dodder, *Cuscuta campestris* Yuncker.

The virus, when tested in fresh cucumber extracts, was found to have a thermal inactivation point of 52°C. for 10 minutes, and a dilution end point of 1:30. Expressed juice kept at room temperatures was infectious for only 12 hours. The virus was found to be still infectious after 142 days when dried in small pieces of cucumber leaf tissue over calcium chloride at 2°C.

In an attempt to extend the herbaceous host range of the virus, a total of 57 plant species representing 20 different families was tested. No herbaceous hosts other than cucumber and squash, *Cucurbita maxima* Duchesne var. Giant Summer Crookneck, were found.

The virus was transmitted mechanically to cucumber from 66 different *Prunus* trees representing 15 species. These trees were known to be infected with a) the necrotic ring spot virus alone, b) latent necrotic ring spot and cherry yellows viruses, or c) recurrent necrotic ring spot and cherry yellows viruses. The symptoms incited in cucumber by each of these 3 distinct sources of inoculum were quite similar. The true identity of the virus responsible for the cucumber disease has not been determined.

In 1939 Keitt and Clayton (6) showed that an unfruitful condition of sour cherry (*Prunus cerasus* L.), then known in Wisconsin as "yellow leaf" or "boarder tree," was actually due to a virus disease, for which they later (7) proposed the common name, cherry yellows. Today cherry yellows is recognized as a major disease of wide occurrence on sour cherry (5).

Moore (11) found that healthy sour cherry trees budded from yellows sources first expressed symptoms completely different from those associated with yellows. These initial symptoms have become recognized as diagnostic of necrotic ring spot, another virus disease. To date the exact relationship of yellows and necrotic ring spot has not been fully established (1, 5). Whereas necrotic ring spot may occur apart from yellows, yellows has never been found free of the necrotic ring spot virus. Ordinarily, striking symptoms of necrotic ring spot are expressed but once, and thereafter the virus is either latent in the infected tree or inconspicuous symptoms are expressed. However, trees infected with necrotic ring spot virus have been found which show striking symptoms every year. These trees are also infected with the cherry yellows virus in all instances studied.

Until recently the only known mode of transmission of these viruses was by grafting, and the known host range was limited to the stone fruits. The discovery by Moore et al (12) that a virus could be mechanically transmitted from yellows infected sour cherry to cucumber (*Cucumis sativus* L.), and from cucumber to cucumber, has however made available other techniques for an expanded study of these diseases.

This paper is a report of investigations which included further work on transmission, physical properties, and host range of the virus transmitted mechanically from cherry, with special reference to the disease incited in cucumber.

MATERIALS AND METHODS.—Cucumber plants were grown in composted soil in 3 in. pots at greenhouse temperatures of 24°–28°C. Large numbers of cucumber plants were required for these studies, and it was found that seeds planted to a depth of ½ in. in vermiculite in clay saucers germinated rapidly and uniformly. Plants transplanted to soil as soon as the hypocotyls had straightened to raise the cotyledons above the level of the vermiculite quickly established themselves, and the cotyledons became well expanded.

[1] Accepted for publication, March 3, 1954.

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Trees were grown in the greenhouse and were handled in a manner found most satisfactory by Moore (11). Transmission of viruses from tree to tree was effected by the chip-bud method. This involved the transfer of a shield of bark, containing a bud, from the scion wood to the stock, from which a shield of bark of similar size and shape had been removed. The transferred buds were held in place by budding rubbers with complete overlapping of the edges of the rubber bands to prevent desiccation.

Preparation of inocula.—Unless otherwise stated, the virus isolates were obtained by thoroughly grinding several young *Prunus* leaves in a sterile mortar with about 2 ml. of distilled water. Usually the leaves were showing necrotic ring spot symptoms (Fig. 1,A), although symptomless young leaves of necrotic ring spot and yellows infected trees were also infectious to cucumber if used within a few weeks after bud break. The virus isolates were maintained in cucumber by serial transfer. In early experiments the *Prunus* or cucumber tissue was ground with 1–2 ml. of 0.1M Na_2HPO_4 buffer with or without 1–2 ml. of 0.01M cysteine hydrochloride (2, 4). In later experiments the tissues were ground with 1–2 ml. of distilled water only.

Method of inoculation.—Unless otherwise stated the cotyledons of cucumber plants were inoculated when the primary leaf measured about ¼–½ in. across. The gauze-pad method of inoculation was employed, and since better results were obtained when the pad was rinsed with water and squeezed out just before using, this procedure was normally followed. Carborundum (600 mesh) powder was sprinkled lightly and evenly over the cotyledons before inoculation. A fine stream of water was used to rinse the plants about 1 minute after the inoculations were made.

CUCUMBER DISEASE INCITED BY VIRUS TRANSMITTED FROM SOUR CHERRY.—Cucumber seedlings inoculated when the primary leaf was just expanding and placed at about 24°C. with adequate light developed small, round, yellow rings on the cotyledons in 2–4 days (Fig. 1,B). These soon became yellow spots which coalesced to form a marked mottle (Fig. 1,C). The cotyledons usually persisted as turgid, functional organs for many weeks in contrast to those of normal plants, which soon withered and died. Within 24–48 hours after symptom development on the cotyledons, yellow spots began to appear on the primary leaf, beginning at the base. Subsequently yellow rings, mottle, and crinkle developed on the affected leaves. Occasionally, the primary leaves withered and died. Usually the apical growing point was killed quickly, and numerous plants were maintained for several weeks with only the 2 cotyledons and the primary leaf. About 30–45 days after inoculation, bud proliferation without elongation was apparent at the node below the killed growing point (Fig. 2, B, C). Many flowers and dwarfed leaves developed in a very compact rosette. In a few instances after a prolonged period of high greenhouse temperatures, several of these severely rosetted plants developed weak, spindly shoots.

Symptoms expressed by cucumber seedlings inoculated when the primary leaf was nearly expanded or later followed a somewhat different pattern (Fig. 3, B). Yellow spots were usually observed on the cotyledons within 3–5 days after inoculation, but the appearance of systemic symptoms did not always follow immediately. Usually the growing points of systemically infected plants were killed by the time the plants were in the fourth or fifth leaf stage, but rarely a plant would continue to grow. Thirty to 45 days after inoculation, bud proliferation without elongation was observed in the leaf axils of some plants where the growing points had been killed. Striking green and yellow mottles developed in the leaves of these systemically infected plants (Fig. 3,A).

TRANSMISSION STUDIES FOR STANDARDIZING TECHNIQUES.—An early effort was made to standardize the inoculation techniques so that all later mechanical transmissions of the virus from cherry to cucumber or from cucumber to cucumber would be made under similar conditions most favorable for maximum disease development. Accordingly, inoculation experiments were made to determine: 1) the age of the cherry leaf tissue from which the virus could be consistently transmitted; 2) the age at which cucumber was most susceptible; 3) the difference in varietal susceptibility in cucumber; and 4) the relation of temperature and light to symptom expression and disease development in cucumber.

Age of cherry leaf tissue and virus transmissibility.—Cucumber plants were inoculated with juice from leaves of sour cherry in varying stages of maturity. The cherry leaves were all showing striking symptoms of necrotic ring spot. It was found that the virus was most readily transmitted from young leaf tissue showing the initial symptoms of necrotic ring spot. Transmission of the virus to cucumber was usually 100 per cent when young (2–3 weeks after bud-break), unfolding leaves from elongating terminals or spurs were used as the source of inoculum. All attempts to transmit the virus from nearly mature leaves showing striking symptoms, with or without necrosis, were unsuccessful. It is possible that these older leaves contain some virus-inhibiting substance that is released and mixed with the virus during the grinding (9). This seems to be a plausible explanation since pieces of these older leaves can transmit virus when placed under the bark of healthy trees.

Age of cucumber and susceptibility.—Using the variety Maine 2, experiments were made to establish the age at which to inoculate plants for a high percentage of infection and consistent symptom development. Groups of 10 plants were inoculated, respectively, 11, 16, 21 and 26 days after seeding. The percentage of infection and disease development were markedly affected by the age of the plants at the time of inoculation (Fig. 2, A). From the data in Table 1, it can be seen that the percentage of infection dropped as the age of the cucumber plants at time

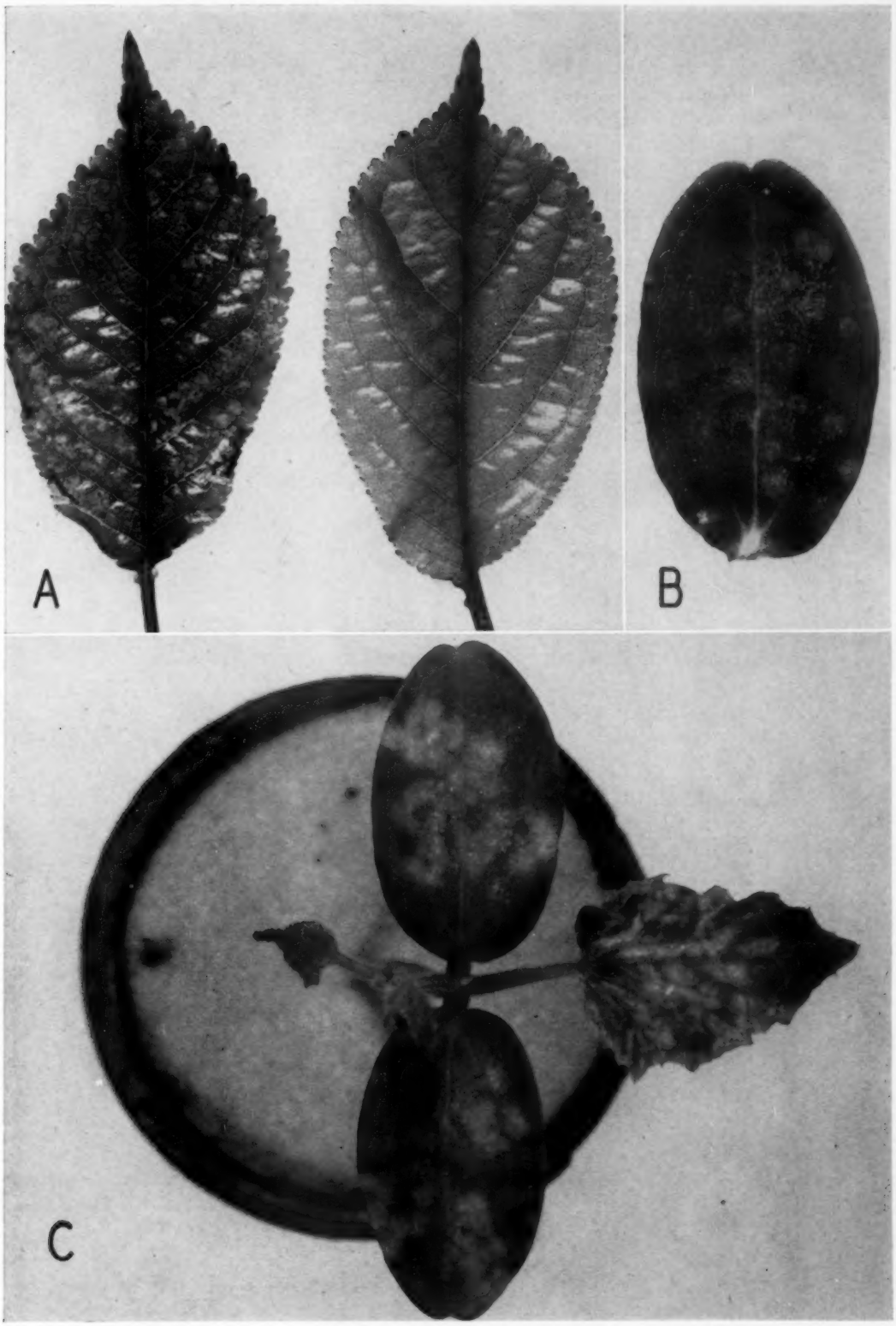

FIG. 1. A) Left, initial symptoms of necrotic ring spot on a Montmorency sour cherry leaf from the greenhouse, tissue at the right age for ready transmission of the virus to cucumber; right, leaf from a control tree. B) Symptoms incited on a Maine 2 cucumber cotyledon by the virus from cherry, 3 days after inoculation. C) Cucumber plant 12 days after inoculation; the yellow spots on the cotyledons have enlarged and coalesced; note killed apical growing point.

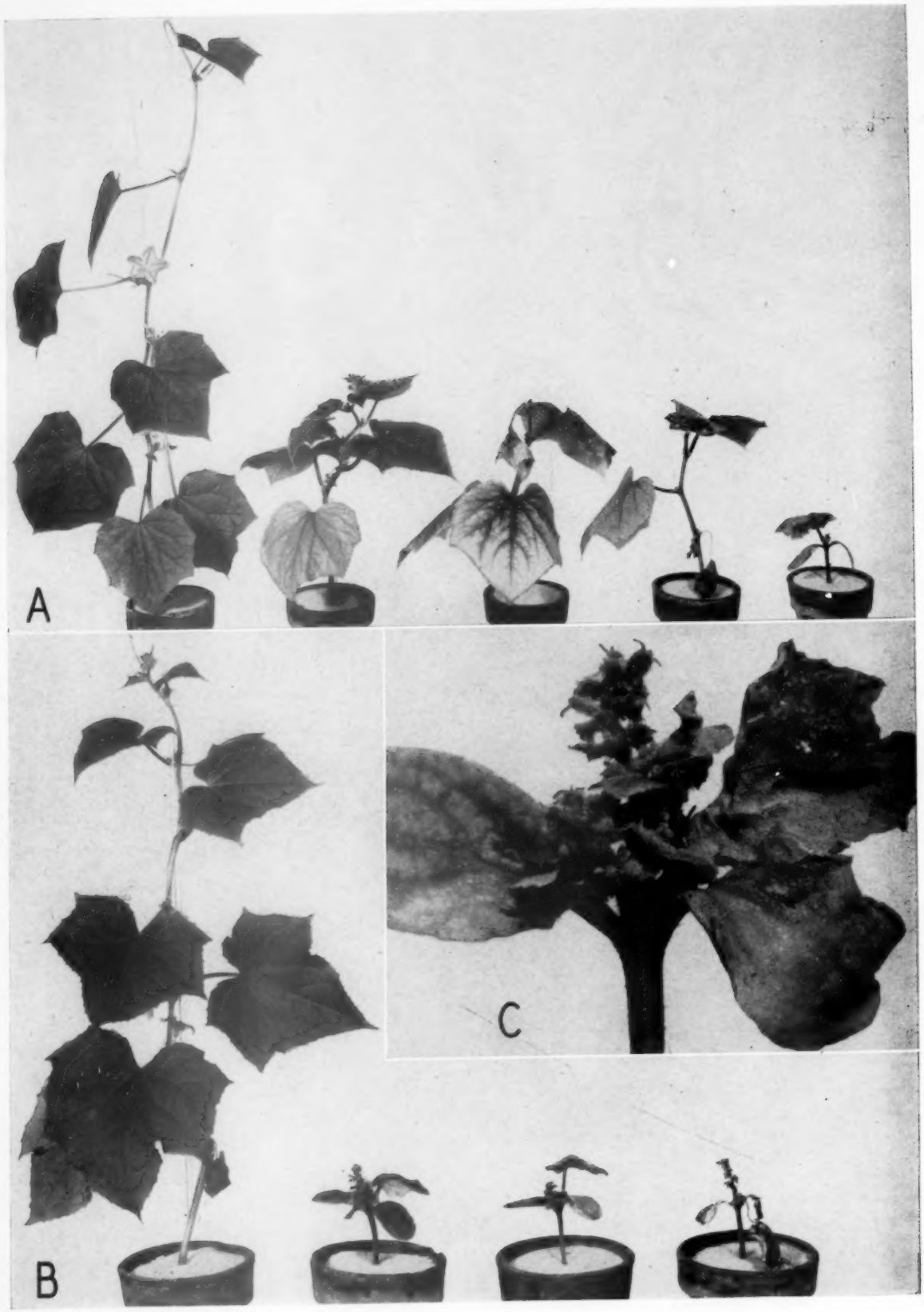
A
B
C

TABLE 1.—*Age of cucumber seedlings at time of inoculation as related to symptom expression and disease development*

Age of cucumbers in days	Stage of development at time of inoculation	Cucumbers infected	
		Cotyledonary symptoms expressed	Systemic symptoms developed
		Number	Number
11	Primary leaf ¼ to ½ inch across	10/10[a]	10/10
16	Second leaf ½ to 1 inch across, third leaf expanding	10/10	2/10
21	Third leaf about 1 inch across, fourth leaf expanding	2/10	2/10
26	Fourth leaf about 1 to 1½ inches across, cotyledons turning yellow	0/10	1/10

[a] Number of plants infected over number of plants inoculated.

of inoculation increased. Additional experiments substantiated these data and, insofar as possible, all later inoculations were made when the primary leaf was just expanding or, at the latest, when this leaf measured no more than ½ in.

Varietal susceptibility of cucumber.—Twenty standard varieties of cucumber were tested for susceptibility. Fifteen plants of each variety were tested as follows: Five plants were inoculated with inoculum prepared from sour cherry leaves showing initial symptoms of necrotic ring spot, and 10 plants were inoculated with inoculum prepared from diseased cucumbers. The cherry leaves used as inoculum were from trees budded with a field source of necrotic ring spot and yellows. The cucumbers used as inoculum had been infected previously from this same field source.

All varieties tested were susceptible. However, based upon the severity of symptoms and percentage of infection, they were divided into 2 classes. The varieties Climbing (Fig. 4, B), Davis Perfect, Early Fortune, Early Green Cluster, Early Russian, Improved Chicago Pickling, Maine 2, National Pickling, Ohio 31, and Select White Spine were the most susceptible. The varieties Ace (Fig. 4, A), Colorado, Cubit, Chinese Long, Improved Long Green (Fig. 4, C), Longfellow, Stays Green, Straight Eight, Tokyo Long Green and White Wonder were least susceptible. The varieties Ohio 31, Tokyo Long Green, and Chinese Long have considerable resistance to cucumber virus 1, but all were susceptible to the virus from cherry. As a result of these experiments, Maine 2 was chosen for further work and used extensively.

Temperature.—It has been shown that temperature is an important and sharply limiting factor in the expression of leaf symptoms of cherry yellows and that these leaf symptoms are masked at constant temperatures of 20°C. and above (8). Symptom expression of necrotic ring spot on sour cherry, on the other hand, has been found to occur over a tested range of 16-28°C. Disease development at the lower temperatures progresses slowly with little or no necrosis. At the higher temperatures disease development progresses more rapidly and with considerable necrosis.

Cucumber plants grown at approximately 24°C. were inoculated with the virus from cherry and placed at 16°, 20°, 24° and 28°C. Symptom expression was delayed at 16° and 20°C., and infected plants at these temperatures showed a tendency to collapse. The 24°C. temperature was near optimum for symptom expression and disease development, and in later experiments when possible, cucumbers were grown and kept at this temperature before and after inoculation. At 28°C. the plants grew rapidly and often the growing point was not killed until the third or fourth leaf stage. Disease development was less severe than at the lower temperatures (Fig. 4).

Light.—It was observed in the spring of 1948 that the percentage of infection of cucumber increased and the symptom expression and disease development became more predictable as the day lengths increased. From June 1 to September 1 almost without exception plants developed striking symptoms. The growing points were killed within a week, and the cotyledons and primary leaves persisted as mottled, functional organs for many weeks. In late fall the percentage and severity of infection varied with each transfer of the virus from cucumber to cucumber. In January, however, it was found possible to duplicate the percentage of infection and the type of symptom expression of midsummer by supplementing the light of the short winter days with artificial light from 6:00 a.m. to 10:00 p.m. These results confirm those of Willison and Weintraub (14) who found significant differences among plants inoculated with the same inoculum but assayed under different environmental conditions. Supplemental light supplied by 200 watt unfrosted bulbs suspended about 20 in. above the greenhouse bench and 2 to 3 ft. apart was sufficient for good symptom expression and disease development in cucumber.

OTHER METHODS OF TRANSMISSION STUDIED.—A number of attempts to transmit the virus from diseased to healthy cucumbers by use of the melon aphid, *Aphis gossypii* Glover, were unsuccessful.

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FIG. 2. A) Age of Maine 2 cucumber at time of inoculation as related to symptom expression and disease development; picture taken 39 days after 11-day-old plants inoculated. From left to right: uninoculated; inoculated when 26, 21, 16 and 11 days old; note start of bud proliferation at node below killed growing point. B) Symptoms on cucumber incited by virus from cherry, 6 weeks after inoculation; control plant on left, note normal elongation of internodes; other 3 plants inoculated with different isolates of cherry virus; note bud proliferation without elongation of internodes. C) Enlargement of an infected plant shown in B.

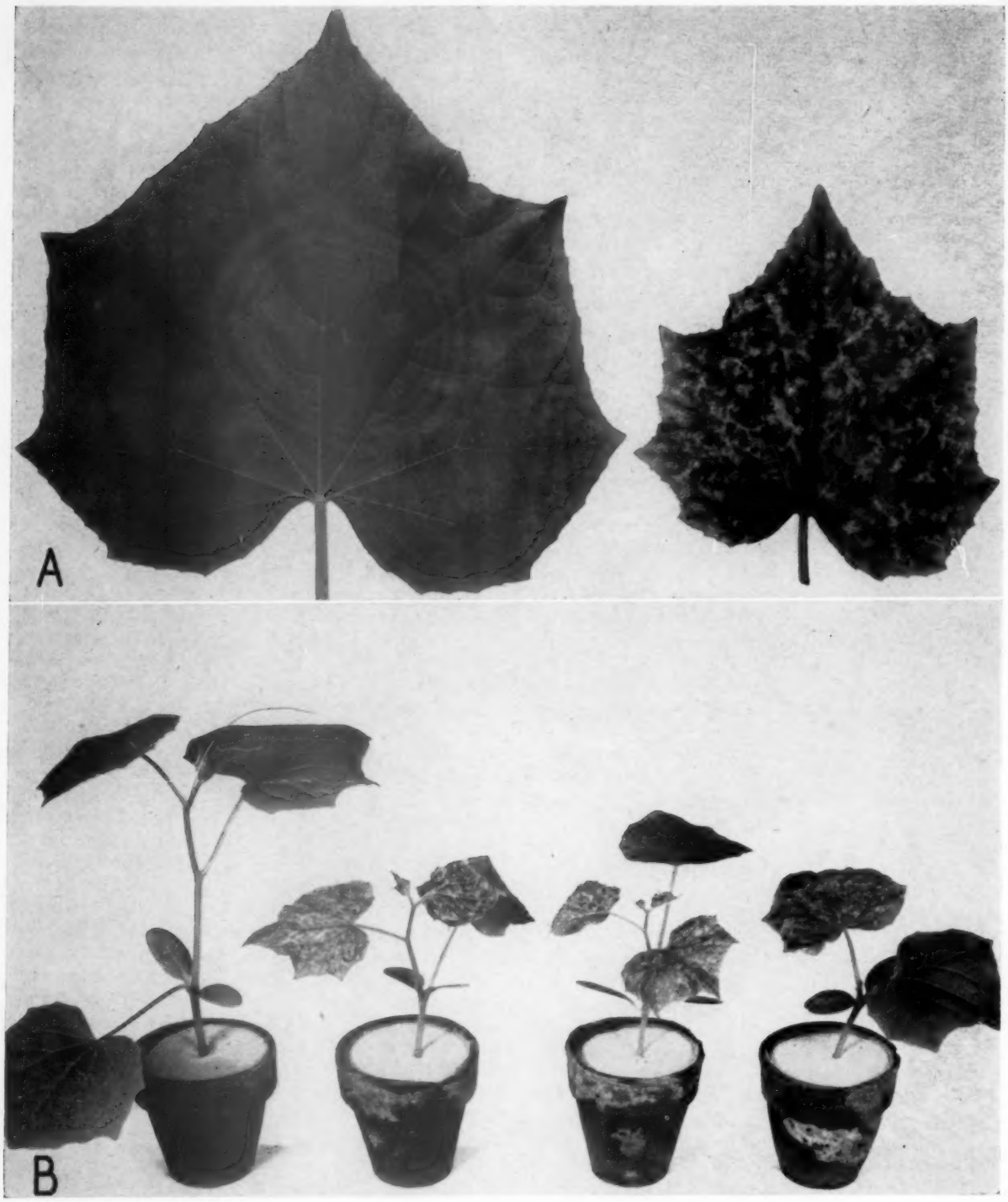

FIG. 3. Systemic symptoms incited in Ohio 31 cucumber 12 days after inoculation with the virus from cherry. A) Left, second leaf from control plant; right, second leaf from infected plant. B) Control plant on left; other 3 plants inoculated with 3 different isolates of the cherry virus when the primary leaf was almost fully expanded.

All attempts to transmit the virus from diseased to healthy cucumbers by use of dodder, *Cuscuta campestris* Yuncker, were unsuccessful.

Only a limited number of cucumber seed was available for study of seed transmissibility, since severely diseased plants seldom matured fruit. How-

ever, a few fruits with mature seeds were collected from diseased plants in late summer. Virus was readily transmitted from the pulp of these fruits. The seeds were dried and planted in vermiculite. Most were sterile, and those seeds that were fertile produced weak and abnormal plants. Two plants were grown for 2½ months and, though they were abnormal in appearance, all attempts to transmit the virus from them to other cucumbers gave negative results.

PHYSICAL PROPERTIES.—The properties of a virus isolate from a necrotic ring spot and yellows source were studied by methods described by Walker et al (13). A preliminary report of this work has been published (3). Extracts from recently infected cucumber plants were used as the inoculum. At least 5 Maine 2 cucumber seedlings were used as a test unit and each experiment was repeated 3 times. The properties of the virus in cucumber extract were as follows: Thermal inactivation point, 52°C. for 10 minutes; inactivation *in vitro* at room temperature, 14 hours; dilution end point, 1:30. It was found that this isolate was much more sensitive to heat, dilution, and aging *in vitro* than all strains of cucumber virus 1 with which it was compared. Willison and Weintraub (15) recently found that stone-fruit viruses isolated from trees affected with prune dwarf, green ring mottle, necrotic ring spot, a recurrent ring pattern, tatter leaf, and cherry yellows were all relatively unstable. These isolates, when compared with respect to their longevity and behavior during aging *in vitro*, varied with the isolate used, the storage temperature of the inoculum, pre- and post-inoculation conditions during the growth of source plants, and dilution.

By use of the technique described by McKinney (10), it was found that the infectivity of the virus from cherry could be preserved for many months in small pieces of cucumber leaf tissue dried in tightly sealed containers over calcium chloride at about 2°C. Young leaves from recently infected cucumber seedlings were used for this study and water extracts prepared from this dried tissue were found to be infectious even after 142 days.

HOST RANGE.—In an attempt to determine in part the experimental herbaceous host range of the virus from cherry, species of plants commonly found in the orchards of Door County, Wisconsin, some cultivated plants susceptible to strains of cucumber virus 1, and some cucurbitaceous plants other than cucumber were grown from seed in the greenhouse. At least 10 young plants of each species were mechanically inoculated with a fresh extract of the cherry virus from recently infected cucumber plants. The infectious nature of the extracts used to inoculate each series of test plants was proved by inoculations to cucumber. After 15–30 days several leaves were taken from each of the 10 or more plants of each species being tested. The leaves from each species were ground together, and the extract was used to inoculate 10 healthy cucumber seedlings.

Under the conditions of these experiments only 1 of the plant species inoculated developed symptoms, and it (*Cucurbita maxima* Duchesne) was the only one from which the virus was transmitted back to cucumber. The following species representing 20 families were not infected: AIZOACEAE; *Mollugo verticillata* L.: AMARANTHACEAE; *Amaranthus graecizans* L.: ASCLEPIADACEAE; *Asclepias syriaca* L.: CARYOPHYLLACEAE; *Cerastium vulgatum L., Lychnis alba* Mill.: CHENOPODIACEAE; *Chenopodium album* L., *Spinacia oleracea* L.: COMPOSITAE; *Anthemis cotula* L., *Arctium minus* (Hill) Bernh., *Centaurea* sp., *Cichorium intybus* L., *Cirsium arvense* (L.) Scop., *Sonchus oleraceus L., Taraxacum officinale* Weber, *Xanthium echinatum* Murr., *Zinnia elegans* Jacq.: CRUCIFERAE; *Brassica arvensis* L., *Capsella bursapastoris* (L.) Medic., *Erysimum cheiranthoides* L., *Hesperis matronalis* L., *Iodanthus pinnatifidus* (Michx.) Steud., *Sisymbrium officinale* (L.) Scop., *Thlaspi arvense* L.: CUCURBITACEAE; *Cucumis melo* L., *Cucurbita pepo* L., *Citrullus vulgaris* Schrad.: EUPHORBIACEAE; *Euphorbia esula* L.: HYPERICACEAE; *Hypericum perforatum* L.: LABIATEAE; *Nepeta cataria* L., *Leonurus cardiaca* L.: LEGUMINOSAE; *Melilotus alba* Desr., *Melilotus officinalis* (L.) Lam., *Phaseolus vulgaris* L., *Pisum sativum* L., *Trifolium incarnatum* L., *Vigna sinensis* (L.) Endl.: MALVACEAE; *Abutilon theophrasti* Medic., *Gossypium hirsutum* L., *Malva rotundifolia* L.: PLANTAGINACEAE; *Plantago major* L.: POLYGONACEAE; *Rumex crispus* L., *Rumex britannica* L., *Fagopyrum esculentum* Gaertn.: PORTULACACAE; *Portulaca oleracea* L.: ROSACEAE; *Potentilla monspeliensis* L.: SOLANACEAE; *Datura stramonium* L., *Lycium halimifolium* Mill., *Lycopersicon esculentum* Mill., *Nicandra physaloides* (L.) Pers., *Nicotiana glutinosa* L., *Nicotiana rustica* L., *Nicotiana tabacum* L., *Physalis pubescens* L., *Solanum nigrum* L.: SCROPHULARIACEAE; *Verbascum thapsus* L., *Chelone glabra* L.

Two separate series of inoculations were made to a number of different varieties in 5 genera of the Cucurbitaceae, and approximately 20 seedlings of each variety were tested. Except for wild cucumber, all seeds were secured from a local seed house and the plants were grown and inoculated in the same manner as cucumber. It was necessary to stratify the wild cucumber seeds before they would germinate.

With the exception of 1 variety of squash, Giant Summer Crookneck, none of the species and varieties became infected. These plants were: *Citrullus vulgaris* Schrad. (watermelon) vars. Dixie Queen, Kleckley Sweet, Stone Mountain; *Cucumis melo* L. (muskmelon) vars. Milwaukee Market, Pride of Wisconsin, Select Osage; *Cucurbita maxima* Duchesne (squash) vars. Buttercup, Early Prolific Straightneck, Improved Hubbard, Italian Marrow, Table Queen; *Cucurbita pepo* L. (pumpkin) vars. Sugar, Dickinson, Winter Luxury; *Echinocystis lobata* (Michx.) T. & G. (wild cucumber).

TRANSMISSION FROM OTHER PRUNUS SPECIES TO CUCUMBER.—The original transfer of the cherry virus to cucumber had been made by using young leaves

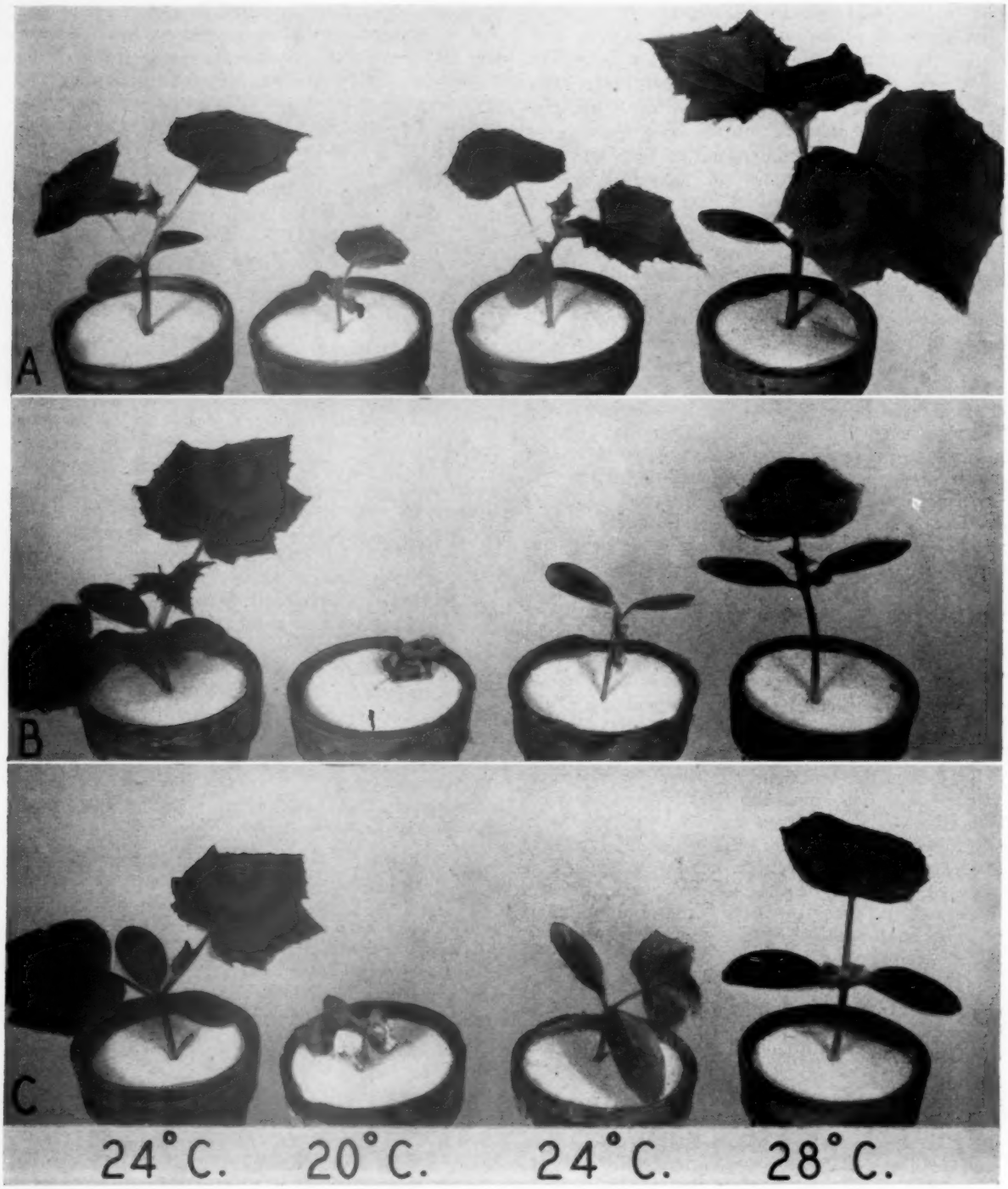

FIG. 4. Susceptibility of 3 different cucumber varieties grown at different temperatures. A) Ace. B) Climbing. C) Improved Long Green. Control on left; other 3 plants in each group inoculated with fresh extracts of the cherry virus.

of sour cherry showing the initial symptoms of necrotic ring spot. This tree, however, had been inoculated from a source carrying both the necrotic ring spot and yellows viruses.

Experiments were made to determine whether the virus was transmissible to cucumber from 1) sour cherry and other *Prunus* species known to be infected with necrotic ring spot only, 2) *Prunus* species other

than sour cherry known to be infected with both necrotic ring spot and yellows, 3) sour cherry and other *Prunus* species known to be infected with the recurrent type of necrotic ring spot and yellows. In these studies young leaves showing symptoms and young leaves without symptoms from trees of each of the above 3 groups were used. Two sources of necrotic ring spot only, 4 sources of latent necrotic ring spot and yellows, and 2 sources of the recurrent type of necrotic ring spot and yellows had been used to inoculate the various species that were tested. Young leaves from necrotic ring spot and yellows-free trees of each species were used for control inoculations.

Juice from leaves of each tree under test was used to inoculate 10 or more cucumber plants. A virus was transmitted to cucumber from 66 trees represented in the following 15 *Prunus* species: *P. americana* Marsh; *P. avium* L.; *P. cerasus* L.; *P. domestica* L.; *P. dunbari* Rehd.; *P. glandulosa* Thunb.; *P. japonica* Thunb.; *P. maackii* Rupr.; *P. mahaleb* L.; *P. padus* L.; *P. pennsylvanica* L.; *P. pumila* L.; *P. serotina* Ehrh.; *P. virginiana* L.; and *P. besseyi* Bailey.

A virus was as readily transmitted from trees infected with recurrent necrotic ring spot and yellows viruses or latent necrotic ring spot virus only, as from trees infected with latent necrotic ring spot and cherry yellows viruses. Symptoms on cucumber infected from different virus sources and from different *Prunus* species were quite similar. Studies are being continued to determine possible strain relationships among these 3 groups.

The true identity of the virus transmitted to cucumber from different *Prunus* species has not been determined, since a method for the consistent transfer of the virus from cucumber back to cherry has not been worked out. Of 6 sour cherry trees inoculated by placing small pieces of diseased cucumber leaf under the bark, 1 developed definite necrotic ring spot symptoms on 2 of the side branches. The following year necrotic ring spot symptoms were expressed on all branches of this tree except the 2 which had shown symptoms previously. Two of 23 pin cherry trees inoculated by placing small pieces of diseased cucumber under the bark showed shock symptoms of necrotic ring spot after about 2 weeks. Studies on the identification of the virus are being continued.

Discussion.—Until 1930 a total of only 5 stone fruit virus diseases, all on peach, had been described in the literature. In the recent U.S.D.A. Agriculture Handbook 10, compiled 21 years later, 48 virus diseases are considered. It is not known how many of these diseases are caused by the same virus or combinations of viruses. There is little wonder that the problem is confused and that the increasing seriousness of the virus problem in stone fruits has become a matter of growing concern to nurserymen, fruit growers, and regulatory agencies.

A serious barrier to investigation of virus diseases of sour cherry has been that the only known mode of transmission was by grafting, and the known host range was limited to the stone fruits. The discovery by Moore et al (12) that a virus can be transmitted mechanically from sour cherry to cucumber has opened possibilities for easier experimentation with this group of virus diseases.

In the present investigation, methods for mechanical transfer of a virus from some *Prunus* species to cucumber have been developed. The virus can be transmitted consistently from *Prunus* to cucumber and from cucumber to cucumber. Consistent mechanical transmission of the virus back to *Prunus* has not yet been accomplished, and the true identity of the virus has not been established. The symptoms of the disease on cucumber differ from those of previously described virus diseases of cucumber. The physical properties and host range of the virus from cherry were found to differ from those of the several strains of cucumber virus 1 with which it was compared.

The limited property studies undertaken in this investigation have shown that the cucumber host affords favorable material. Diseased tissues are readily produced in sufficient quantity and yield satisfactory extracts.

The property studies of the virus from cherry were made with cucumber plants inoculated from a sour cherry tree budded from a source carrying both the necrotic ring spot and yellows viruses. It will be desirable to make comparative studies of the virus from additional sources including trees carrying necrotic ring spot only and those carrying the recurrent type of necrotic ring spot. The materials now available might afford opportunity to supplement property studies by application of such techniques as serology and electron microscopy.

Of the herbaceous plants tested, only the cucumber varieties and the squash variety Giant Summer Crookneck were found susceptible to the virus from cherry; however, with modified techniques it is quite possible that some plants that gave negative results might be infected. The fact that this virus has been successfully transmitted to cucumber and squash suggest the desirability of more extended search for herbaceous plants that may serve as hosts and reservoirs for stone fruit viruses.

It is not known how extensively cucumber can be used in the study of the stone fruit virus diseases. However, since it has proved such a good host in these studies, it would seem to warrant consideration either as a possible herbaceous host or as a differential herbaceous host for other stone fruit viruses. Furthermore, it may well be considered as a possible indexing plant for the necrotic ring spot virus.

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HETEROTHALLISM IN PHYTOPHTHORA PARASITICA VAR. NICOTIANAE [1]

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SUMMARY

As a result of mating studies, in various combinations, among 35 single zoospore isolants of *Phytophthora parasitica* var. *nicotianae* from black shank affected tobacco in Kentucky it was found that 2 cultures were antheridial and 33 were oogonial.[2] Oospores, with amphigenous antheridia, averaging 23.15 microns, developed only when an antheridial strain was paired with an oogonial strain, and then only on agar containing fresh green tobacco leaf extract.

The appearance of black shank at different times in widely separated areas in Kentucky, its occurrence after 20 years and at a great distance from Georgia where the disease was first reported in the U. S., the comparatively slow build-up of the disease to destructive proportions, the inability to attribute many new outbreaks to the introduction of the pathogen by the usual methods (water, man, tools, etc.) suggest the independent origin of the pathogen, in new isolated outbreaks, by hybridization or otherwise from *Phytophthora* already present (11). That there are different strains of the pathogen, presumably of different origin, and that hybridization occurs between different isolants of the black shank pathogen is shown in the studies herein reported.

As a result of mating studies of different species of *Phytophthora*, Ashby (1), Gadd (3), Narasimhan (7), and Leonian (5) concluded that there was sufficient evidence for the occurrence of heterothallism. Lester-Smith (6), in similar studies, concluded that the formation of oospores could be explained biochemically but Narasimhan (7) and Leonian (5) believed that this view failed to explain why oospores are produced only when certain strains are paired. Tucker (10) suggests that more studies are necessary to establish the occurrence of heterothallism in *Phytophthora*.

Phytophthora parasitica var. *nicotianae* Tucker, originally described in 1896 by Breda de Haan, the causal organism of black shank of tobacco, is reported

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[2] Since this paper was accepted for publication, 6 cultures of the black shank fungus have been received from North Carolina and 1 from Virginia, all of which were antheridial; and 1 culture from East Tennessee, which was oogonial.

as rarely producing oospores in culture. Jensen (4), Tisdale and Kelley (9), Rosenbaum (8), and Lester-Smith (6) failed to obtain oospores. Tucker (10), who found an occasional oospore in old cultures, stated that oogonia were observed by Breda de Haan, Lodewijks, and Ashby.

Experimental results.—Single zoospore cultures of *P. parasitica* var. *nicotianae* Tucker were prepared from each of 35 isolants from tobacco from 35 different Kentucky fields affected by black shank. Preliminary studies with some of these at 30°C. indicated that oospores resulted when certain strains were mated on 1½ per cent agar containing 25 ml. per liter of fresh tobacco extract. No oospores developed when matings were made on oat, Czapek's, or plain water agar.

Matings among the 35 single spore cultures were made in many different combinations. It was early observed that oospores resulted only when either 1 of 2 strains, 1120 or 1127, were used in the matings. Oospores never developed when 1120 was mated with 1127 nor when either was plated alone.

Culture 1120 was mated with the other 33 single zoospore strains and oospores developed in every mating. All 33 strains were plated alone and in various combinations with each other, but in no case did oospores develop.

Culture 1127 was paired with the same 33 cultures that were paired with 1120. Oospores resulted in only 11 of the pairings. It is not known why oospores did not develop with the other 22 isolants.

Both 1120 and 1127 often produced antheridia in culture when grown alone but always produced them when oospores were produced in matings. These studies indicate that cultures 1120 and 1127 are antheridial, and the other 33 cultures are oogonial.

Oospores (Fig. 1) have amphigenous antheridia and range in size from 20 to 32 microns, with an average of 23.15 microns. These measurements compare favorably with those given for *P. parasitica* var. *nicotianae* by Ashby (2) and Tucker (10). A few oospores formed germ tubes, at room temperature, after having been kept at freezing for 12 hours. One germ tube formed a typical sporangium.

Fig. 1. Oospore of *Phytophthora parasitica* var. *nicotianae* showing amphigenous antheridium. ×970.

Discussion.—These studies indicate that antheridial and oogonial strains of *P. parasitica* var. *nicotianae* are present in nature and that these strains are capable of hybridization with all the variation that this implies. *P. parasitica* var. *nicotianae*, which differs from *P. parasitica* only by its ability to cause black shank in tobacco, may have arisen many times from the latter by hybridization.

The results suggest that at least 4 different strains of the fungus are present in Kentucky; 2 distinct antheridial strains, one of which mated with 33 oogonial strains and the other with only 11 strains; and at least 2 oogonial strains, one group of which hybridized with cultures 1127 and the other group which failed to hybridize with it. These 4 strains suggest independent origin of the fungus parasitic on tobacco on at least 4 occasions.

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DETECTION OF VIRUS IN CATTLEYA ORCHIDS BY SEROLOGICAL METHODS[1]

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SUMMARY

Virus in *Cattleya* orchid plants has been detected by utilizing anti-virus antisera in interfacial ring tests. Virus, purified by differential centrifugation from plants showing symptoms of the flower-breaking disease of *Cattleya* orchids, was used as antigen to induce the formation of anti-virus antibodies in rabbits, and the antisera were employed as reagents to test leaf-tip extracts of other orchid plants for presence of virus. Tests performed with these sera detected virus in symptomless plants as well as in those showing symptoms.

Two proteins have been shown to be associated with the *Cattleya* flower-breaking disease by electrophoretic analysis and electron microscopy.

Positive identification of plants suspected of being virus-infected is often difficult because the symptoms of some physiological disorders may be mistaken for, or mask, symptoms associated with virus infection. Typical of this situation is the flower-breaking disease of *Cattleya* orchids which has been attributed to a virus by Jensen (7), who was able to transmit the disease by means of an aphid. Gold (5) using the electron microscope, has identified rod-like particles in an extract from virus-infected orchid plants, and this work has since been confirmed by Newton and Rosberg (11), who also found that extracts from virus-diseased plants were able to induce the formation of anti-bodies in animals.

Based upon our present knowledge of this disease, the unequivocal diagnosis of virus in a plant has rested on either transmission trials to other orchids or electron microscopy of leaf extracts as suggested by Newton and Rosberg. Both methods are obviously difficult for the grower to employ on a large scale. On the other hand, use of anti-orchid virus antiserum in a simple laboratory test, as described below, is an inexpensive and practical means for screening large numbers of orchid plants for presence of virus. Chester (3) first utilized serological techniques in a test designed to identify 6 viruses of tobacco in the field, and this approach to plant virus diagnosis has later seen limited application with other viruses and hosts. The orchid is very suitable for commercial application of the serological method, as each plant is of sufficient worth to warrant an individual test.

Development of the method for detection of virus in *Cattleya* orchids takes advantage of the fact that viruses are high molecular weight nucleoproteins which often can be detected in the presence of normal host tissue by physical methods (15) and may thereby be separated from these normal components.

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MATERIALS AND METHODS.—*Plant material.*—Most of the *Cattleya* leaf material used in this study was kindly donated by Armacost and Royston Inc., Los Angeles, California, and their cooperation is gratefully acknowledged. Leaves were taken, in the main, from older plants capable of producing flowers.

Preparation of virus.—Virus was extracted from orchid leaves by a method only slightly modified from that of Stanley (14). Distilled water was used as the extracting solvent, and the virus preparation obtained after 3 cycles of alternating high and low speed centrifugation in a Spinco Model L centrifuge was dialyzed for 24 hours against distilled water at 4°C. before use.

Electrophoresis. — The virus preparations were examined electrophoretically using methods and equipment recently described (12). The following buffers were used: pH 6.9 cacodylate; pH 6.9 cacodylate adjusted to pH 5.8 with 0.1 N HCl; and pH 5.1 acetate buffer. Eighty per cent of the ionic strength of each buffer was donated by NaCl. The protein concentrations of virus analyzed ranged from 0.2 to 0.5 per cent.

Electron microscopy.—Preparations to be examined with the electron microscope were mixed with a small amount of polystyrene latex solution (Dow Chemical Co. 580 G, Lot 3584) which served as a marker in the microscope field. The virus-polystyrene preparation, with an approximate virus concentration of 0.01 mg. virus per ml., was directly applied in a drop to formvar coated screens, and kept in a moist chamber for 12 hours. After drying in air, the screens were shadowed with uranium and examined with an RCA Model EMU electron microscope.

Nitrogen and virus concentrations.—Nitrogen determinations were made by the standard Nesslerization procedure. Virus concentrations are calculated figures, the product of the nitrogen determination times 6.25.

Trichloroacetic acid precipitations.—Virus or *Cattleya* normal protein material was precipitated by following the method of Eggman et al (4).

Ultra-violet absorption.—The ultra-violet absorption spectrum of a virus preparation was obtained with a Beckman Model DU spectrophotometer. The virus, present in a concentration of 0.1 mg. per ml., was suspended in 0.1 ionic strength cacodylic acid buffer.

Preparation of the anti-virus antiserum.—The purified virus was used to induce formation of antibodies in 4 Dutch breed rabbits weighing about 5 lbs. each. The virus, diluted with phosphate buffer to a concentration of 1 mg. per ml., served as injection antigen. The first rabbit was injected intravenously with 1 ml. and then with doubling volumes at successive 2-day intervals (2, 4, and 8 ml.), so that the animal received a total of 15 mg. of virus. The second rabbit was injected with a total of 9 mg. of virus in doses of 1, 2, and 6 mg. In later immunizations, a third animal received 8 mg. and a fourth 19 mg. in similar doses. Three to 11 days after the last injection, blood was removed from the marginal ear vein or from the heart of each rabbit. The length of time, in days after the final virus injection, for the antibody titre to reach a maximum in each animal immunized was ascertained by determining the relative antibody content of a small amount of serum obtained by a daily test bleeding from the ear. The daily relative titer, as indicated by means of the floculation time (2) observed with standard dilutions of purified virus, determined the bleeding date for the individual animal. The animals were bled once each day for 2 consecutive days following the day on which the antibody titer reached its maximum. The blood was allowed to clot for several hours at room temperature, and the serum removed by centrifugation. The serum from the 2 bleedings of a given rabbit was pooled, but the serum from each of the 4 rabbits was stored and used separately. Normal, or non-immune, serum was obtained from the blood of these animals taken prior to the injection of any antigen.

Serological techniques.—The quantitative precipitin analysis was performed by the technique of Heidelberger and Kendall (9). Both normal serum and antiserum were diluted 1:4 with 0.9 per cent saline.

Antigen-antibody reactions in agar followed the modified Oudin technique as described by Munoz and Becker (10). The sera to be tested were mixed with agar such that the final agar concentration was 0.3 per cent and the sera were diluted 1:2 and 1:4. *Cattleya* proteins employed in this technique were prepared from virus-free leaves (determined by serological test) following the scheme described by Wildman and Jagendorf (16) for the preparation of leaf cytoplasmic proteins. To this protein preparation was added 10 per cent of an aqueous solution of merthiolate (1:1000) before it was layered onto the serum-agar mixtures contained in glass tubes, 5mm. in diameter.

EXPERIMENTAL RESULTS.—*Nature and properties of the virus.*—The protein extracted from diseased *Cattleya* leaves exhibits properties which are typical of high molecular weight, rod-shaped, plant virus nucleoproteins. The material is stream double refracting in polarized light, and has a very slow rate of diffusion in an electrophoretic cell. It contains pentose (qualitative orcinol test), and, as can be seen in Fig. 1, its ultra-violet absorption spectrum, showing an absorption maximum at 260 mμ, closely resembles that of other plant virus nucleoproteins (1). Furthermore, all the nitrogen contained in the virus can be precipitated with 1 N trichloroacetic acid.

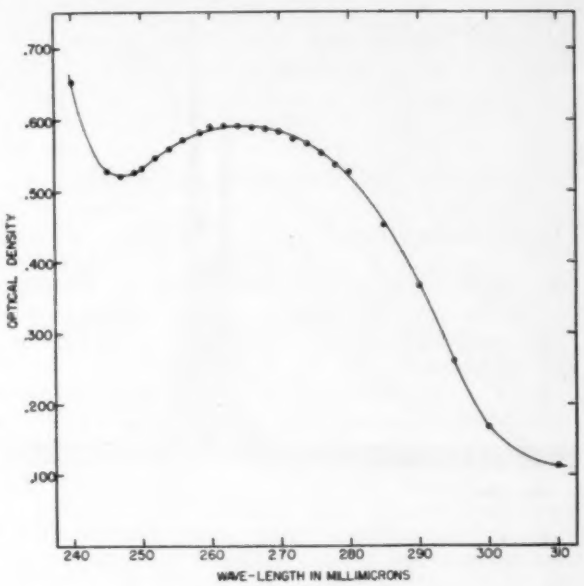

FIG. 1. Ultra-violet absorption spectrum of *Cattleya* orchid virus.

Preparations of the purified virus were also examined in the electron microscope. The 2 distinct types of rods observed by previous workers (6, 11) were evident in all preparations examined.

When subjected to electrophoretic analysis at pH 6.9 the virus migrated as a single sharp spike with little tendency for the boundary to spread. However, at the trailing edge of the spike there exists a shoulder which suggests the presence of an additional component in the preparation. Electrophoresis at pH 5.8 or 5.1 resolved the virus preparation into 2 sharp spikes as shown in Fig. 2; the relative amount of each component in the mixture may be different from preparation to preparation, but in each case, the leading component showed the greater concentration. The average mobility of the major component of purified orchid virus is -8.2×10^{-5} cm/sec/volt/cm in 0.1 ionic strength cacodylic acid buffer, pH 6.9.

Antiserum studies.—The antiserum is most conveniently stored by maintaining it in the frozen state (−10°C.). One serum thus preserved has maintained anti-virus activity after storage for 2 years. During this time it had been thawed and refrozen several times. It is also possible to maintain the serum at 2-4°C. for several weeks. If this is done it is necessary to add 10 per cent of an aqueous solution of merthiolate (1:1000) to inhibit the growth of bacteria and molds.

The normal (non-immune) sera exhibit a non-specific reaction, when used without prior dilution, in

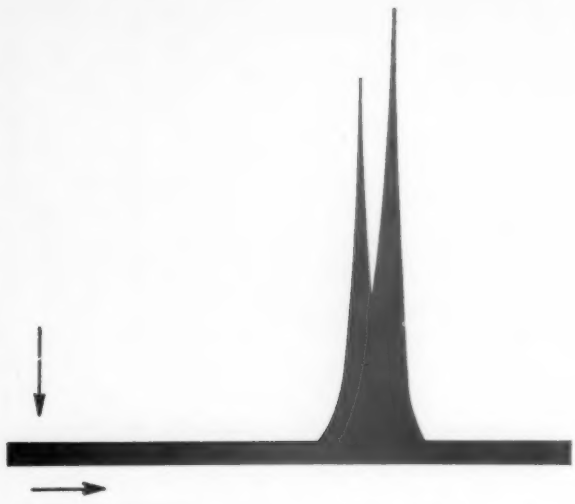

FIG. 2. Electrophoretic pattern (ascending boundary) of *Cattleya* orchid virus. Sixty minutes migration at a current of 10 milliamperes in 0.1 ionic strength cacodylic acid buffer, pH 5.8.

an interfacial ring test with the purified orchid virus. The normal serum effect may be eliminated through the use of antisera and normal sera diluted 1:4 or greater in saline. We have found that the minimum dilution of 1:4 is best for plant testing work. The sera, when diluted 1:4, react in a ring test with concentrations of purified orchid virus greater than 0.02 mg. per ml.

To eliminate the possibility of the sera containing antibodies for contaminating host proteins, the virus antisera were tested by the modified Oudin technique against a preparation of host proteins. The cytoplasmic protein preparations used in the test contained 0.21 mg. nitrogen per ml., 80 per cent of which was precipitable with trichloroacetic acid. Except for 1 antiserum which gave a doubtful reaction, all sera were negative in the test. Only those antisera which showed no reaction with normal leaf proteins were used in the subsequent tests.

As seen in the electron microscope and confirmed electrophoretically, the virus protein extracted from *Cattleya* is a mixture of 2 viruses. It would be valuable in a study of this type to know if antibodies are formed to one or both of these viruses, in order to define more clearly the ability of the serum to detect each virus in the mixture. The different electrophoretic mobility of the 2 viruses at pH 5.8 (and in more recent work at pH 5.1 in acetate buffer) suggests that it is possible to separate physically the viruses from an electrophoretic cell (13). This would be useful in obtaining sera to each virus individually, such that the qualitative nature of the virus present in a given plant could be determined. Unfortunately, the low yields of virus obtainable from *Cattleya* plants have prevented separation of the viruses in quantities sufficient for the formation of antibodies. However, a quantitative precipitin analysis with 1 antiserum showed that more than 1 species of antibody was present. Both antigen and antibody were detected in the supernatant of 1 of the precipitin reaction tubes. It is well established (9) that this situation can exist only when the antigen-antibody system is complex; i.e., when it contains more than 1 antigen and more than 1 antibody. In the light of the information obtained by means of the Oudin test showing that no antibodies are formed against normal *Cattleya* proteins, we interpret these results to mean that there are antibodies in this serum to more than 1 virus.

Plant testing procedure.—The following procedure has been utilized to screen orchid plants for the presence of virus. A small portion (approximately 1 sq. in.) of the tip of a *Cattleya* leaf was removed and frozen in a deep freeze. The sample was pulverized in a mortar while still frozen and then ground to a pulp with 5–8 times its weight of pH 7, M/15 phosphate buffer to which had been added 0.01 M Na diethyldithiocarbamate (Eastman #2596), and a small amount of washed sand to aid in the grinding. The presence of the carbamate in the buffer prevents the formation of a dark brown color which can often obscure the observation of the serological ring test.

To the extract, filtered through coarse filter paper, was added ½ volume of chloroform to aid in the removal of pigments and interfering host plant substances. This mixture was agitated for ½ minute and centrifuged at 4°C. for 20 minutes at 3500 r.p.m. in an International Size 1 centrifuge (#811 head). A small amount of the upper, water phase was removed with a pipette and used in a conventional ring test by carefully layering it over an equal amount of the antiserum (diluted 1:4 with 0.9 per cent saline) contained in a 3 in. length of 4 mm. glass tubing held in a rack with the base of the tube inserted into plasticene clay. For each sample a control tube, containing normal serum layered in the same manner, was used for comparison.

When virus was present in the extract, the formation of a white precipitate at the antigen-antibody interface occurred within 15-20 minutes. Only reactions which occurred within 20 minutes are considered positive because of non-specific reactions which develop slowly with longer periods of time.

Application of the antisera in plant tests.—To test the validity of the serological method for routine screening of individual orchid plants for presence of virus, 28 plants were examined. A qualified observer classified 6 of the plants as definitely containing virus, 8 which showed symptoms suggestive of virus as doubtful, and the remaining 14 plants as healthy. A tissue extract was made from a portion of each plant and tested with the anti-virus antiserum. Complete agreement was reached in that plants showing symptoms of the virus also reacted with the antiserum, whereas symptomless plants failed to do so. With the doubtful cases, 7 out of 8 plants gave a positive serological reaction.

As a further test of the reliability of the serological method as a means for detecting virus-infected plants, arrangements were made to apply the test to a larger number of plants. The aid of an outside observer was enlisted who collected leaf tip samples from 99 individual orchid plants. In contrast to the previous sample of 28 plants, the samples were selected at random from a number of different orchid growing establishments in this area. After selection by the observer the grower was requested to diagnose the plant as healthy or virus diseased. In some instances the grower considered himself unable to render an unequivocal diagnosis, and such plants were indicated as doubtful. After recording the grower's opinion, the leaf tips, identified by numbers only, were tested individually with the anti-orchid virus antiserum. When it is considered that opinions of 5 different orchid growers were used and that there were no means of confirming the diagnoses based on symptoms, the over-all agreement with the serological results may be considered good. Of the 90 plants diagnosed by the growers as definitely healthy or definitely virus diseased, 69 per cent of these diagnoses were confirmed by serological test. The doubtful plants are not included in this figure because of the reluctance of the grower to state his opinion; however, 7 of the 9 plants gave a positive serological reaction for virus.

Of the total 28 disagreements, 15 were considered virus diseased by the growers but failed to give a positive serological reaction. Six of these 15 plants came from one grower, 4 from another, 3 from another, and 1 each from 2 other growers. We are inclined to believe that these disagreements arose from the difficulty in recognizing the presence of virus from leaf symptoms, perhaps as the result of physiological or pathological disorders mimicking virus symptoms.

The remaining 13 disagreements consisted of plants classified as healthy by the growers but contained virus according to the serological test. Five of the 13 plants showed leaf symptoms of some sort, but the growers were not prepared to classify them as typical of the flower-breaking disease. Nevertheless, the growers requested that they be subjected to the serological test since symptomatological diagnosis was uncertain. The fact that the plants gave positive serological reactions suggests either a variation in the virus symptom pattern or perhaps symptoms of the non-viral origin superimposed on the virus symptom pattern.

Eight of the 13 plants were considered by the growers to be free from virus, as they exhibited no symptoms of any kind, yet gave a positive reaction in the serological diagnosis. It is likely that these plants are symptomless carriers of the virus, or new infections which have not yet developed overt symptoms of the disease. Support for this interpretation comes from a consideration of 2 of these plants. One plant was derived by vegetative propagation from a plant in which identification of virus was definite by both symptoms and serological test. Similarly, the other leaf sample represented a symptomless leaf from a plant which showed symptoms of virus on other leaves as well as a positive serological reaction. Thus, the serological method seems capable of detecting virus infection before the onset of visible symptoms of the virus disease.

Investigations on symptomless plants.—Extracts of 3 symptomless *Cattleya* flowers from plants (not of the group of 99 plants discussed above) which showed no leaf symptoms, but which gave positive serological tests were examined under the electron microscope for the presence of rods associated with virus infection. Similar extracts were also prepared from flowers of 3 symptomless plants which gave a negative serological test, as controls. To encourage the maximum opportunity to observe rods, the extracts were highly concentrated—using only 1 cycle of high and low speed centrifugation in preparation.

Under the electron microscope the extracts from the plants which were positive serologically showed an uncountable number of rods in microscope fields containing 4–12 polystyrene balls at magnifications of approximately 10,600 times. In the control extracts, fields which contained the same number of polystyrene balls as markers contained no rods, thus offering the assurance that a positive serological reaction is directly correlated with the presence of rods, regardless of whether the infection has produced symptoms typical of the disease.

Discussion.—On the basis of the above results, we may conclude that the serological method is a simple means for detecting virus in *Cattleya* orchids. The virus preparations used as immunizing antigens show 2 distinct morphological types of rods, and electrophoretically show 2 components. Since the negative Oudin tests rule out the possibility of antibodies reacting with healthy leaf proteins, the lack of a clear equivalence zone in the quantitative precipitin analysis indicates that the antisera react with both viruses.

Gold and Jensen (6) have demonstrated that either or both morphological types of rods may be found in *Cattleya* plants showing symptoms of the flower–breaking disease. One of these viruses is transmissible to *Cymbidium* and is the virus which is associated with *Cymbidium* mosaic. The other virus is easily transmitted to and extracted from *Odontoglossum grande*, and is the cause of a ring spot in that plant. Our serum prepared from *Cattleya* virus has enabled us to diagnose successfully leaf samples of *Odontoglossum* ring spot, kindly sent to us by Dr. Jensen. Some samples of *Cymbidium* obtained locally, which showed symptoms of the *Cymbidium* mosaic virus were also tested, and the presence of *Cymidium* mosaic virus in them was confirmed with anti-*Cattleya* virus antiserum. From this evidence we feel that the serum we have produced contains antibodies to at least these 2 viruses, although the exact proportion in the serum of each of these is not known. Consequently, a plant classified as virus diseased in our investigation most

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likely is infected with either the *Cymbidium* mosaic virus, the *Odontoglossum* ring spot virus, or both. Either or both of these viruses may cause the plant to show broken flowers, according to Gold and Jensen (6).

The outstanding contribution of the serological testing method in a virus-control program would be in the screening of *Cattleya* plants in order to detect those virus-diseased plants with absent or obscure leaf and flower symptoms. As Jensen (8) has pointed out, "failure to date to cure virus-infected orchid plants makes the control problem one of disease prevention rather than cure." Preventing the spread of the virus, whether by insects or mechanically, depends upon: 1) The positive identification of virus-infected plants; 2) removing the source of virus inoculum by destroying or isolating the virus-infected material. Use of anti-virus antiserum in mass screening of individual orchid plants presents a means whereby virus-infected plants could be positively identified. Healthy plants set apart from the diseased, and kept free from virus-carrying insects appears to be a logical method of controlling this disease.

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A COMPARATIVE STUDY OF DIAPORTHE STEM CANKER AND POD AND STEM BLIGHT OF SOYBEAN [1]

Kirk L. Athow and Ralph M. Caldwell [2]

SUMMARY

A comparison is presented of Diaporthe stem canker and pod and stem blight of soybean and of the organisms responsible for these 2 diseases.

Diaporthe stem canker is an acute disease causing death of actively growing plants as a result of rapid development of stem girdling lesions. The casual organism of Diaporthe stem canker is here recognized as a variety of *Diaporthe phaseolorum* under the trinomial *Diaporthe phaseolorum* var. *caulivora* and a technical description of the variety is presented.

Pod and stem blight is a slow developing disease which occasionally causes slightly premature ripening and a discoloration of a small portion of the pith of infected plants. It is characterized by linear rows of pycnidia on mature, dead, or moribund stems, petioles, and pods. The trinomial, *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehm. is retained as the valid name of the fungus causing the pod and stem blight disease of soybean.

Diaporthe phaseolorum var. *caulivora* is differentiated from *D. phaseolorum* var. *sojae* by its lack of a conidial stage, the production of perithecia in caespitose groups instead of singly, shorter and more tapering perithecial beaks, and smaller asci and ascospores.

Stem canker is one of the most destructive diseases of soybean (*Glycine max* (L) Merrill) in north central United States. Historically this disease has been considered as one symptom of the pod and stem blight disease of soybean. Consequently, *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehm. was recognized as the causal organism until Welch and Gilman (16) reported that the stem canker inducing fungus differed from it in morphology and pathogenicity. They suggested that the stem canker fungus was a strain of *Diaporthe phaseolorum* var. *batatatis* (Harter & Field) Wehm. In the study reported here a detailed comparison was made of the 2 apparently different pathogens involved. This paper presents the pathological and morphological findings that serve as a basis for ascribing a new varietal name, *Diaporthe phaseolorum* var. *caulivora*, to the fungus associated with the stem canker disease.

MATERIALS AND METHODS.—Stem canker and pod and stem blight were observed in the field and numerous isolates of the stem canker and pod and stem blight pathogens were studied. The different isolates of each organism were similar except for abundance of sporulation. For this reason most of the comparisons were made with 1 representative isolate of each organism. One culture of *D. phaseolorum* var. *sojae* from soybean in Georgia was obtained from E. S. Luttrell. Isolates of Diaporthe from sweet potato, presumably *D. Phaseolorum* var. *batatatis*, were obtained from H. Rex Thomas in Maryland and V. M. Held in New Jersey.

The most commonly used media for comparing types of fructifications and spore forms were 2 per cent potato dextrose agar, sterile soybean stems, and steamed whole oat, rye, and sorghum grain. The media were seeded with mycelia from single spore cultures. Measurements were made of fruiting bodies and spores produced on several of the media and on the host. Dimensions reported are means of at least 100 measurements.

The pathogenicity of the cultures was studied by inoculating soybean plants in the greenhouse and field. Inoculations were made by inserting mycelium growing on PDA into a wound in the stem and by the "toothpick method" (4) which proved to be the more satisfactory. Inoculations, in most cases, were made in a leaf scar at the first, second or third node. The highly susceptible soybean variety Hawkeye was used in all the studies of pathogenicity.

SYMPTOMS.—*Stem canker.*—The earliest symptom of stem canker is a reddish-brown discoloration of a leaf scar (Fig. 1B, 1C) or, less frequently, of the pulvinus of a petiole at one of the lower nodes. A definite lesion (Fig. 1D) develops from this initial point of infection. The slightly sunken canker frequently attains a length of several inches and may completely encircle the stem. Stages in lesion development are shown in Fig. 1E. Girdled plants (Fig. 1F) become conspicuous in the field. The dead leaves remain on such plants after healthy plants have shed their leaves. Lesions have not been observed on pods or leaves.

Pod and Stem Blight.—Pod and stem blight is characterized by linear rows of pycnidia on stems (Fig. 2A), petioles, and pods (Fig. 2B). Leaf blade infection has not been observed by the writers, although it has been reported by Lehman (12) and

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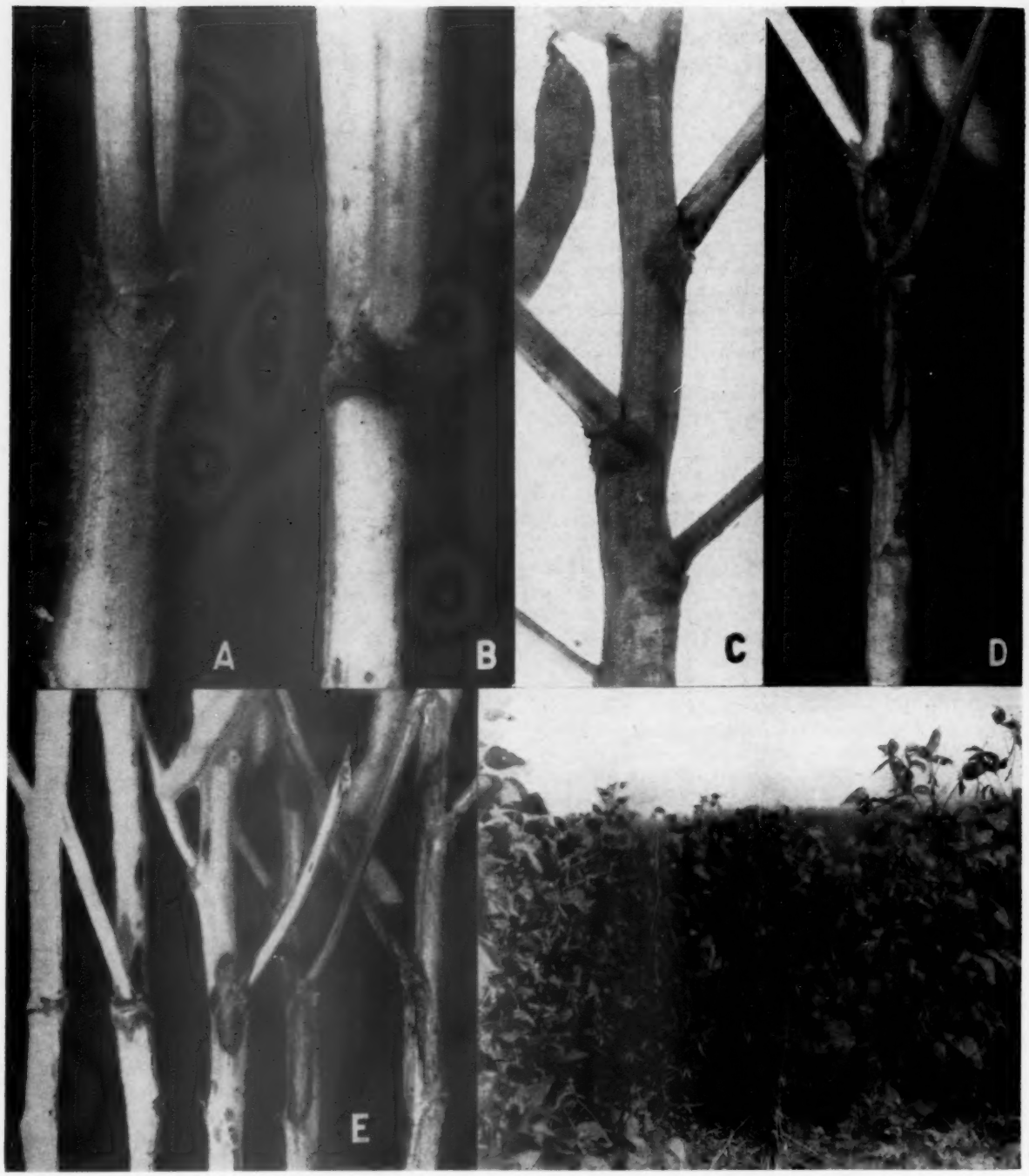

FIG. 1. Symptoms of Diaporthe stem canker on soybeans. A) Healthy leaf scar. B) Early infection. C) Several infected leaf scars. D) Girdling lesion. E) Various stages of lesion development. F) Plants killed by stem canker.

Luttrell (13). The disease first appears on the petioles of lower leaves and upon broken lower branches. Pycnidia develop on the main stem and upper branches after the death of the entire plant following maturity or death from other causes. Dead stems may be covered with pycnidia or the pycnidia may be limited to small patches usually near the nodes. Pod and stem blight under natural conditions produces no definite lesion such as is caused by stem canker. Pycnidia frequently are found on the dry,

FIG. 2. Symptoms of pod and stem blight on soybean stem (A) and pod (B).

poorly developed pods. Proof is lacking that the fungus is the cause of the failure of the pods to develop. Excessive podding, environmental conditions, or attack by other organisms may be the primary cause of the death of immature pods.

ECONOMIC IMPORTANCE. — *Stem Canker.* — Stem canker is of major economic importance because of its ability to attack and kill actively growing plants. Soybean fields in Indiana have been observed in which 25-40 per cent of the plants were killed. The extent of loss depends upon plant maturity at the time of infection. Natural infection has never been observed before the plants were at least 70 days of age. Yield is greatly reduced if plants become infected at this age when the pods are just beginning to fill. Comparisons of seed from 100 healthy and 100 plants killed by stem canker in each of 2 fields of Hawkeye soybeans showed approximately a 60 per cent reduction in seed weight from the diseased plants. This figure represents a conservative estimate of the usual loss, as most of the uppermost pods were beginning to fill when the plants were killed. Furthermore, this does not take into account the loss in seed quality or of the small, shriveled seed which would be lost in the threshing and cleaning processes.

Pod and Stem Blight.—Pod and stem blight is of little or no economic importance as is implied by the description of symptoms.

DISTRIBUTION.—*Stem Canker.*—The disease is known to occur in Iowa (3, 16), Illinois (1, 10), Indiana, Ohio, Michigan[3], Minnesota (11), and Ontario, Canada (8). It is most prevalent in east central Iowa, central Illinois and central Indiana and has not been found in southern Indiana or Illinois. It has been impossible to determine from the literature whether the disease occurs in other soybean growing regions because of the common practice of applying the name pod and stem blight to any Diaporthe disease on soybean. However, in a report of pod and stem blight in Virginia, Fenne (5) mentioned girdling of stems and premature death of plants. Stem canker may be more wide spread but this cannot be established until the disease is more generally recognized.

Pod and Stem Blight.—Pod and stem blight is found in all the soybean producing areas in the United States. The disease also has been reported from Canada (2, 9) and Japan (14, 18). It is assumed that most of the references are only to pod and stem blight, as here recognized, because no mention is made of girdling lesions, the typical symptom of stem canker.

HOST RANGE.—*Diaporthe phaseolorum var. caulivora.*—The stem canker fungus has been found on or reported only from soybean. Artificial inoculation of lima bean and tomato by the writers has not resulted in infection.

Diaporthe phaseolorum var. sojae.—The pod and stem blight organism has been reported on soybean, snap bean, cowpea, lima bean, peanut, lupine, lespedeza, *Strophostyles helvola*, tomato, pepper, okra, onion, and garlic. Luttrell (13) reports that as a wound parasite it is capable of causing a rot of pepper and tomato fruits. It appears to be a weak parasite on the other hosts.

MORPHOLOGY.—*Diaporthe phaseolorum var. caulivora.*—The perithecia of *D. phaseolorum* var. *caulivora* develop on overwintered stems. They are black, globose, and occur in caespitose groups of 2–12, predominantly 3–5 per stroma. Occasionally a single perithecium is found. The perithecial dimensions are 165-340 × 282-412 μ. The perithecia are embedded in cortical tissue. The protruding perithecial beaks vary in length from 85 to 192 μ in width at the base to 22-36 μ in width at the tip and from 24-518 μ in length. The 8-spored asci are sessile, elongate-clavate, with thin evanescent walls slightly thickened at the apex.

[3] Personal observations in Indiana, Ohio, and Michigan.

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The asci measured 29.8-40.2 × 4.5-7.0 μ. The ascospores are elongate-ellipsoidial, bicellular, slightly constricted at the septum, and possess 2 guttulae per cell. They measured 8.6-11.8 × 3.0-3.9 μ. The ascospores germinate within 4–6 hours from one or both ends of the spore.

Single spore isolates produce visible growth on PDA within 3 days and completely cover a 100 mm. Petri plate within 10 days at room temperature. The mycelium at first is closely appressed, but within a few days becomes flocculent with typical mycelial knots scattered over the surface. The mycelium is white and the agar medium remains unpigmented.

Mycelium develops abundantly on rice, soybean, sorghum, soybean agar, and corn meal mush, but very few perithecia are produced. A sparser mycelium with more perithecia develops on sterile soybean stems, steamed whole oats or rye, and on cooked, rolled wheat. The perithecia produced in culture are slightly larger than those on the host. An imperfect stage of *D. phaseolorum* var. *caulivora* has never been found either on the host or in culture.

Diaporthe phaseolorum var. sojae.—The perithecial stage of *D. phaseolorum* var. *sojae* was found much less frequently on overwintered stems than that of *D. phaseolorum* var. *caulivora*. The perithecia, borne singly in a black stroma in the cortical tissue, measured 148-282 × 185-346 μ and had long, tapering beaks. The perithecial beaks range from 60-142 μ in width at their base and from .35 to 1.5 mm. in length. The asci are 8-spored, sessile, elongate-clavate, with thin evanescent walls slightly thickened at the apex. They measured 38.0-51.2 × 5.0-10.3 μ. The ascospores are hyaline, elongate-ellipsoidal, bicellular, slightly constricted at the septum, with 2 guttulae per cell. The spores measured 9.2-13.5 × 3.3-5.6 μ.

In contrast to *D. phaseolorum* var. *caulivora*, *D. phaseolorum* var. *sojae* has pycnidia which constitute the conspicuous fruiting form. The pycnidia on the stems and petioles of the host develop in compact groups in a black, pulvinate stromata beneath the epidermis. The pycnidia measured 112-542 × 98-385 μ. The pycnidia usually contain a single cavity, although several cavities may occur. The pycnidia contain conidia and sometimes both conidia and stylospores. The conidia, borne on short, simple, unicellular conidiophores are unicellular, hyaline, fusiform-elliptical, with 2–4, predominantly 2, guttulae. The spores measured 4.9-9.8 × 1.8-3.2 μ. The stylospores, which were found only on the host, are hyaline, filiform, usually curved structures and measured 14.1-35.1 × 1.2-1.7 μ. The conidia germinate readily but the stylospores have not been germinated.

Cultures from ascospores and conidia are indistinguishable. Cultures grown from single spores are visible within 3 days and completely cover a 100 mm. Petri plate within 1 week with a light gray, flocculent mycelium. Yellow and green areas frequently occur in the cultures and all cultures produce a definite darkening of agar media. Pycnidia develop in cultures of most isolates within 2–3 weeks. None of the isolates of *D. phaseolorum* var. *sojae* have produced perithecia on any of the cultural media employed. The isolate of *D. phaseolorum* var. *sojae* from Georgia was similar to isolates from Indiana in all cultural and morphological characteristics studied.

Attempts to induce the formation of perithecia or to demonstrate homo- or hetero-thallism on potato dextrose agar or steamed soybean stems have been unsuccessful. These experiments were duplicated and involved 1 group of 10 ascosporic cultures in 1949 and a second group of 29 ascosporic cultures in 1950 within which all possible paired combinations were made. Welch and Gilman (16) using similar technique demonstrated heterothallism with Iowa cultures.

Diaporthe sp. from Sweet Potato.—Neither of the 2 isolates of Diaporthe from sweet potato produced perithecia in culture so it was impossible to be certain that they were actually *D. phaseolorum* var. *batatatis*. However, their conidial stages were similar to those described by Harter and Field (6) and Wehmeyer (14) for this organism. Both isolates produced pycnidia abundantly on all media on which they were grown. The pcynidia were scattered singly on the cultures rather than occurring in groups in pulvinate stromata as were the pycnidia of *D. phaseolorum* var. *sojae*. The pycnidia measured 65-154 × 58-128 μ. The conidia were unicellular, hyaline, fusiform-elliptical, 2–4, predominantly 2, guttulate, and measured 5.7-10.2 × 2.8-4.2 μ. Filiform, curved stylospores ranging from 15-33 × 1.1-1.9 μ were produced in abundance. Also, a smaller spore form occurred regularly in cultures of these 2 isolates. They were cylindric, club-shaped, usually curved, and measured 6.4-14 μ in length. They were similar in shape and size to the intermediate type spore recorded by Wehmeyer (15) for *D. phaseolorum* var. *batatatis*.

PATHOGENICITY.—*Diaporthe phaseolorum var. caulivora.*—Preliminary inoculations were made in the greenhouse in 1948 and 1949 with a number of isolates of *D. phaseolorum* var. *caulivora*. Plants from 28–86 days old were inoculated by inserting mycelium in stem wounds. There was no appreciable difference in pathogenicity among isolates, but the data suggest that the older plants were the more susceptible. Lesion development on most of the younger plants appeared to be restricted as if by the formation of wound cork.

During the spring of 1950 additional inoculations were made in the greenhouse using cultures of Diaporthe from sweet potato as well as isolates of *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *sojae* from soybean. Inoculations in test I were made by inserting mycelium into wounds and in test II by the toothpick method (Table 1, part A). Thirteen of the 15 plants inoculated with *D. phaseolorum* var. *caulivora* in the 2 tests were killed within 15 days after inoculation. The same cultures were used in making 2 series of inoculations by the toothpick method in the field (Table 1, part B). Of the 75 plants inoculated with *D. phaseolorum* var. *caulivora*, 62 became infected and 41 were killed.

TABLE. 1. *The pathogenicity of Diaporthe isolates from soybean and sweet potato on Hawkeye soybeans grown in the greenhouse and field*

Culture number	Culture and source[a] Organism	Host	Age of plants when inoculated, days		Number of Plants Inoculated		Infected		Killed	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
	A. Greenhouse inoculations									
51	D. *phaseolorum* var. *sojae*	Soybean	78	90	5	9	4	8	0(4)[b]	0(1)
52	D. *phaseolorum* var. *sojae*	Soybean	78	90	6	9	6	7	0(6)	0(2)
53	D. *phaseolorum* var. *caulivora*	Soybean	78	90	6	9	6	9	6	7
56	Diaporthe sp.	Sweet Potato	—	90	—	8	—	1	—	0
57	Diaporthe sp	Sweet Potato	—	90	—	9	—	0	—	0
Check			78	90	6	9	0	0	0	0
	B. Field inoculations									
51	D. *phaseolorum* var. *sojae*	Soybean	71	103	46	45	13	26	0	0
52	D. *phaseolorum* var. *sojae*	Soybean	71	103	36	32	13	6	1	0
53	D. *phaseolorum* var. *caulivora*	Soybean	71	103	44	31	39	23	23	18
56	Diaporthe sp.	Sweet Potato	71	103	31	39	1	0	0	0
57	Diaporthe sp	Sweet Potato	71	103	34	40	0	0	0	0
Check			71	103	40	40	0	0	0	0

[a] Culture 51 was from Indiana, 52 from Georgia, 53 from Indiana, 56 from New Jersey, and 57 from Maryland.
[b] Plants were killed approximately 10 days before the check plants were mature.

Diaporthe phaseolorum var. sojae.—The results presented in Table 1 show that culture 51 from Indiana and culture 52 from Georgia were both weak parasites. In the greenhouse some of the inoculated plants died 7–10 days before uninoculated check plants reached maturity and linear rows of pycnidia developed on the stems of these plants. This appeared to be a hastening of maturity rather than abrupt killing in 15–20 days as in the case of plants inoculated with *D. phaseolorum* var. *caulivora*. For this reason data on pathogenicity were recorded 2–3 weeks after inoculation and again approximately 1 week before the check plants were ripe. The latter figures are in parentheses.

Non-killing lesions developed on the stems of a number of plants inoculated with *D. phaseolorum* var. *sojae*. They were black rather than reddish-brown as in stem canker, and were confined to one side of the stem immediately above and below the point of inoculation. There was no tendency for the lesion to encircle or girdle the stem. A dark discoloration of the pith extending several inches beyond the external lesion was observable when infected stems were dissected. At maturity, linear rows of pycnidia developed on the stems of plants showing non-killing lesions. This indicated that the fungus was able to survive in the host apparently without injuring it appreciably and to produce pycnidia after the plant had ripened.

Diaporthe sp. from Sweet Potato.—Neither of the isolates of Diaporthe from sweet potato was pathogenic on soybean (Table 1). A very limited number of atypical lesions developed on soybean plants inoculated in the field with culture 56. The lesions were small and restricted to the immediate area of inoculation. The isolates produced little or no internal discoloration of the stem tissue as occurred when plants were inoculated with *D. phaseolorum* var. *caulivora* or *D. phaseolorum* var. *sojae*.

TAXONOMY AND NOMENCLATURE.—On the basis of the above characteristics the organism causing stem canker is recognized as a new variety of *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. distinct from *D. phaseolorum* var. *sojae* (Lehman) Wehm. and *D. phaseolorum* var. *batatatis* (Harter and Field) Wehm. The description of the proposed variety, *Diaporthe phaseolorum* var. *caulivora*, is as follows:

Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. *caulivora* var. nov.

Pycnidia et conidia nulla. Perithecia caulicola, globosa, 165–340 × 282-412 μ in stromata nigricantibus laxe aggregata, immersa, ostiolis longis attenuatis, 240-518 × 58-192 μ; ascis elongato-clavatis, sessilibus, octosporis, membrana adlatere tenui ad apicem incrassata, 29.8-40.2 × 4.5-7.0 μ. Ascosporea hyalinae, elongato-ellipsoidae, 1-septatae, ad septum leniter constrictae, cellulis biguttulatis, 8.6-11.8 × 3.0-3.9 μ. Hab. In caulibus *Glycine max* (L) Merrill, Lafayette, Indiana, May 28, 1950 Athow (type).

Type specimens have been deposited in the Arthur Herbarium of the Purdue University Agricultural Experiment Station and in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland.

The pod and stem blight organism reported in this study is similar in most respects to the single perithecial fungus which Welch and Gilman (16) recognized as *D. phaseolorum* var. *sojae*, the perithecial and non-perithecial strains of *D. phaseolorum* var. *sojae* reported by Luttrell (13), and *D. sojae* described by

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Lehman (12). They apparently are closely related strains of a single fungus and it is proposed that they be recognized as *D. phaseolorum* var. *sojae*.

DISCUSSION AND CONCLUSION.—Previous to 1933, the species of the genus Diaporthe were separated mainly on host relationship. Wehmeyer (12) in 1933, monographed the genus Diaporthe and used morphological characteristics rather than pathogenicity as a means of species separation. He combined those species with elongated ostiolar necks under the name *D. phaseolorum* (Cke. & Ell.) Sacc. and based the species on the form described by Harter in 1917 on lima bean. *Diaporthe batatatis* described by Harter and Field on sweet potato in 1912 and *D. sojae* described by Lehman in 1923 on soybean were recognized as *D. phaseolorum* var. *batatatis* (Harter & Field) Wehm. and *D. phaseolorum* var. *sojae* (Lehman) Wehm. respectively on the basis of host relationship.

Luttrell (13) in a study of *D. phaseolorum* and perithecial and non-perithecial strains of *D. phaseolorum* var. *sojae* found that positive separation depended upon differences in pathogenicity and appearance in culture. However, these differences were considered sufficient to retain *D. phaseolorum* var. *sojae* as a variety.

In 1948, Welch and Gilman (16) reported 2 varieties of *D. phaseolorum* on soybeans in Iowa which differed in pathogenicity and in perithecial development. One was heterothallic with scattered single perithecia and the other was homothallic with caespitose clusters of perithecia. The former produced typical Phomopsis conidia of the alpha and beta (stylospore) types; the latter lacked conidia. They recognized the heterothallic form as *D. phaseolorum* var. *sojae* and the homothallic form as a strain of *D. phaseolorum* var. *batatatis*. The latter variety actively attacked soybean stems, girdling them and causing the plants to wilt and die. The former variety was less pathogenic, mainly attacking mature plants and producing linear rows of pycnidia on branches and stems.

The writers are in agreement with Welch and Gilman (16) that 2 distinct diseases caused by morphologically different forms of Diaporthe occur on soybeans but do not find that the stem canker fungus is identical with *D. phaseolorum* var. *batatatis*.

The perithecia of the stem canker fungus are more globose than those of *D. phaseolorum and D. phaseolorum* var. *sojae* but the caespitose habit apparently is not unique with this fungus and *D. phaseolorum* var. *batatatis* (12, 15). The asci of the stem canker fungus are more nearly the same size as those of *D. phaseolorum;* being smaller than the asci of the variety *sojae* and larger than those of the variety *batatatis*. The ascospores of the stem canker fungus are larger than those reported for *D. phaseolorum* var. *sojae* but fall within the range reported for both *D. phaseolorum* and *D. phaseolorum* var. *batatatis*. The perithecial beaks of *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *batatatis* are much larger and less tapered than the perithecial beaks of the stem canker fungus. In this respect the stem canker fungus resembles more closely *D. phaseolorum* although its beaks are slightly longer and more tapered. Thus, the stem canker fungus does not resemble *D. phaseolorum* var. *batatatis* as described by Harter and Field (7) and Wehmeyer (15) in these characteristics more closely than it does *D. phaseolorum* or *D. phaseolorum* var. *sojae*. Furthermore, if the stem canker fungus is considered a strain of *D. phaseolorum* var. *batatatis*, then 2 pathogens, one on soybean and one on sweet potato, differing in morphology and pathogenicity would be recognized as *D. phaseolorum* var. *batatatis*.

The absence of a conidial or imperfect stage offers a distinct morphological basis for separating the stem canker organism from *D. phaseolorum*, *D. phaseolorum* var. *sojae*, and *D. phaseolorum* var. *batatatis*. This characteristic possibly is sufficient to warrant specific recognition of the fungus. However, for the present we elect to follow Wehmeyer's (15) criteria whereby the length of the ostiolar necks and size of spores place the stem canker fungus in that group with elongate beaks for which *D. phaseolorum* is recognized as the type species. The conservative view is therefore chosen and it is proposed that the stem canker fungus be recognized as a variety of *D. phaseolorum* under the trinomial *D. phaseolorum* var. *caulivora*.

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EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF DOWNY MILDEW OF LIMA BEAN [1]

R. S. Cox [2]

SUMMARY

Initial penetration of host tissues apparently occurred directly through the cuticle. Symptoms failed to develop on carbohydrate-deficient plant parts; carbohydrate content appeared to be affected by time of day the pods and leaves were removed from the plant, and by the temperature at which they were subsequently incubated.

Where sporangia-inoculated material was incubated at constant temperatures (°C.) the following were noted: 10°, no disease; 15°, symptoms after 8 days, maximum severity after 10; 20°, the optimum, symptoms after 4 days, maximum severity after 6; 25°, symptoms occurred only once on one pod; 30°, no disease.

Where zoospores were used for inoculum, no disease developed at 10° or 30°C.; it occurred initially after 8 days at 15°; after 4, at 20°; and after 2½, the optimum, at 25°C.

Using various combinations of daily exposure periods at 2 alternating temperatures, disease developed after 2½ days on sporangia-inoculated leaves when successively incubated at 15°C. for 6, and 25°C. for 18 hours; other combinations were less favorable.

In a previous study by Hyre and Cox,[3] it was found that various growth phenomena of the fungus, *Phytophthora phaseoli* Thaxt., which causes downy mildew of lima bean, are very sensitive to certain environmental factors. From the observed effect of temperature on sporangia and zoospore germination and on germ-tube growth, the following inferences could be drawn: a) disease could be expected, though delayed, at 10°C. (50°F.) regardless of inoculum used, since both sporangia and zoospores germinated at that temperature, the latter followed by appreciable germ-tube growth; b) a constant temperature of 30°C. (86°F.) would prohibit disease development regardless of spores used, since germ-tube elongation appeared to come to a standstill soon after the zoospores germinated; c) direct germination of sporangia would not play an important role in disease development at any temperature, since it rarely exceeded 1 per cent; d) using sporangia for inoculum and a constant temperature, maximum disease development would be expected to occur in the neighborhood of 20°C.; little to no disease would be expected at a temperature of 25°C. or higher, since indirect germination rarely occurred at those temperatures; e) starting with zoospores for inoculum, the incidence and rate of disease development would be expected to increase with a rise in temperature through 25°C.; f) finally, maximum disease development would be expected under natural conditions of fluctuating temperatures somewhere around the range 15-25°C. (59-77°F.).

As a first step in evaluating the validity of these inferences, studies were undertaken on the effect of controlled temperature on disease development.

MATERIALS AND METHODS.—The detached plant-part technique[4] was used exclusively. Trifoliate leaves and pods were collected from greenhouse-grown Henderson Bush lima bean plants about 4:00 P.M. on a

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[2] Associate Professor of Plant Pathology, University of Delaware, Newark, Delaware.

[3] Hyre, R. A., and R. S. Cox. 1953. Factors affecting viability and growth of *Phytophthora phaseoli*. Phytopathology. **43**: 419-425.

[4] Yarwood, C. E. 1946. Detached leaf culture. Bot. Rev. **12**: 1-56.

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TABLE 1. *Effect of constant temperatures on disease development on detached leaves.*

Temperature °C.	Inoculum	Disease severity after specified days[a] 2½	4	6	8	10	21
10	Zoospores	—	—	—	—	—	—
	Sporangia	—	—	—	—	—	—
15	Zoospores	—	—	—	+	++++	++++
	Sporangia	—	—	—	+	++++	++++
20	Zoospores	—	++	++++	++++	++++	++++
	Sporangia	—	+	+++	++++	++++	++++
25[b]	Zoospores	+	++	++	++	++	++
	Sporangia	—	—	—	—	—	—
30	Zoospores	—	—	—	—	—	—
	Sporangia	—	—	—	—	—	—

[a] Minus (—) equals no disease, 4 plus (++++) equals maximum symptoms and sporulation.
[b] Where pods were used instead of leaves at 25°C. the following is noted: in the case of zoospore inoculum, the disease progressed rapidly to maximum severity (++++); with sporangia, 1 of 10 tested became diseased after the third day and rapidly developed maximum symptoms and signs (++++) thereafter; thus, initial disease appearance is considered more significant than ultimate severity.

clear day.[5] Various techniques were employed so as to preserve the normal green state of the test material and, at the same time, obtain maximum disease. The most effective method was to suspend the leaf blades above the liquid substrate with the petioles immersed.[6] Circular discs of paraffin-dipped[7] screen wire with the edges bent down were fitted into the moist chamber so that they were raised above the liquid surface. The leaf petiole was fitted through an opening of the screen wire into the liquid. When pods were used, satisfactory results were obtained by floating them on the liquid.

Sporangia and zoospore water-suspensions were prepared for inoculum as described elsewhere.[3] Material was inoculated by pipetting 10 small drops of the desired suspension, each containing several hundred spores, onto the plant part. The moist chambers were incubated in non-lighted temperature units accurate to 0.5°C., except the 10° unit which was more variable.

A total of 8 tests were conducted using the constant-temperature technique and 4 tests using a fluctuating temperature. Each treatment consisted of 4 replications.

EXPERIMENTAL RESULTS.—*Penetration.* — Symptoms were readily obtained on inoculated pods and leaves incubated in continuous darkness. This suggested initial direct germ-tube penetration. In limited studies, direct penetration of zoospore germ-tubes was observed through the cuticle of leaves which had been killed 48 hours after inoculation, cleared in chloral hydrate, and examined in toto following the method of Peace.[8]

Effect of substrate and temperature on longevity and on disease development.—Leaves and pods deteriorated rapidly when floated on or suspended above water. Longevity increased markedly on sucrose solutions up to 10 per cent; further increase in concentration had little effect. As noted by Waters,[6] use of non-sterile sucrose lessened the problem of contamination. No symptoms or signs developed when water was used as a substrate, some were obtained when 4 per cent sucrose was used, maximum, when 10 per cent sucrose was the medium. The significance of the carbohydrate content of host tissues in respect to disease development is well known.[4]

Leaf chlorosis appeared very quickly at the higher temperatures (within 3 days at 30°C. and 5 days at 25°C.); whereas, at 10°C. the material remained green for at least 3 weeks. Pod chlorosis did not appear until after the fourth or fifth day at 30°C. In no case was disease observed after chlorosis became evident.

Effect of constant temperature on disease development.—All tests gave comparable results except that better disease development occurred on pods than on leaves at 25°C. In all cases symptoms were obtained only when young actively-growing plant material was used. This is in agreement with field observations that mature plant tissues (leaves, stems, and pods) are highly resistant to the downy mildew fungus. Results of a representative test are in Table 1.

From these data it can be seen that no disease developed at 10° or 30°C. It appeared after 8 days at 15°, 4 days at 20°, and 2½ days at 25°C. Using sporangia, optimum temperature for disease appeared to be 20°C.; it occurred rarely (only once) at 25°C. With

[5] These tests were conducted during the winter months of 1951-52. It was found that plant parts deteriorated rapidly when collected during the forenoon or at any time on a heavily-overcast day, and that disease development was either inadequate or failed entirely. These phenomena were believed to be tied in with starch deficiency of the plant parts.

[6] Waters, C. W. 1928. The control of teliospore and urediniospore formation by experimental methods. Phytopathology **18**: 157-213.

[7] The paraffin coat prevented rusting and eliminated contact injury of the leaf blade against the metal wire.

[8] Peace, L. M. 1910. Notes upon clearing and staining of leaves and stems. Plant World **13**: 93-96.

TABLE 2.—*Effect of fluctuating temperatures on disease development on detached leaves inoculated with sporangia*

Temperature (°C.) Sequence	Disease severity after specified days[a] 2½	3	4	5	6	8	10
10, constant	—	—	—	—	—	—	—
15, constant	—	—	—	—	—	—	++
20, constant	—	—	+	+++	++++	++++	++++
25, constant	—	—	—	—	—	—	—
30, constant	—	—	—	—	—	—	—
10, 16 hrs.; 30, 8 hrs.[b]	—	—	—	—	—	+	+
10, 18 hrs.; 15, 6 hrs.	—	—	—	—	—	—	—
15, 6 hrs.; 25, 18 hrs.[c]	+	++	+++	+++	+++	+++	+++
20, 20 hrs.; 25, 4 hrs.	—	+	++	++++	++++	++++	++++
20, 20 hrs.; 30, 4 hrs.	—	—	+	+	++	++	++
20, 16 hrs.; 30, 8 hrs.	—	—	+	+	++	++	++
20, 6 hrs.; 30, 18 hrs.	—	—	—	—	—	—	—

[a] Disease ratings same as footnote a, Table 1.
[b] Symptom development but no sporulation.
[c] Disease development progressed rapidly to the 3 plus (+++) stage, then ceased probably due to adverse effect of the higher temperatures on the host tissues. Initial disease appearance is therefore considered more significant than ultimate severity.

zoospores, 25°C. appeared optimum, particularly on pods.

Effect of fluctuating temperatures on disease development.—To more nearly simulate field conditions, a series of tests were conducted in which sporangia-inoculated leaves were held successively at 2 different temperatures for pre-determined periods of time. This was accomplished by setting up a duplicate series of moist chambers, 1 chamber at each temperature used in a particular combination. At the desired time daily the screens containing the leaves were exchanged between the 2 chambers. By having the pre-conditioned moist chambers in each unit and interchanging only the wire screens and leaves, the time lag in adjustment of host tissues to the current temperature was considerably reduced. Results of a representative test are in Table 2.

Although neither 10° nor 30°C. alone favored the disease, the data show that a certain combination of exposure periods at these temperatures could result in symptom development. Also, exposure at 30°C. for as little as 4 hours of each 24 over a 10-day period curtailed disease development; 18 continuous hours exposure daily prevented it entirely. Symptoms appeared only after 2½ days at the 15°-25°C. combination, the most favorable one tested.

DISCUSSION.—Data herein reported carried previous basic information[3] one step closer to field conditions, and, in general, confirmed the inferences formulated. One deviation from the expected was the failure of disease to develop on leaves or pods held continuously at 10°C. Waters[6] observed the formation of teliospores at lower temperatures on plants in the greenhouse than on detached leaves floated on sucrose solution. He attributed this to inability of detached leaves to form starch at low temperatures. In the present work, inoculated leaves that had been held previously at 10°C. for 2 weeks developed symptoms within 2 days after being transferred to 20°C. It might be deduced from this observation that infection occurred at 10°C., but symptoms were masked.

The rapid deterioration of host tissues, particularly leaves, exposed to continuous high temperatures also undoubtedly adversely affected disease development, and should be taken into consideration in interpretation of results. Intermittent exposure to these high temperatures had less adverse effect.

It is significant that daily exposure periods of as short a duration as 4 hours at 30°C. (86°F.) appeared to reduce considerably disease development. Thus, epiphytotics might not be expected under field conditions where the daily maximum exceeded that temperature. It is also suggested that certain critical periods of minimum temperatures in the neighborhood of 20°C. (68°F.) or less are essential for disease development in the field, and that the temperature range most conducive to the development of an epiphytotic is somewhere around 15-25°C. (59-77°F.). This information though not considered conclusive should aid in the development of a disease forecasting system.

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Longevity of the Oak Wilt Fungus in Diseased Trees.[1] EDWARD L. MEREK AND CHARLES L. FERGUS.[2] The longevity of *Endoconidiophora fagacearum* Bretz in various parts of diseased trees has been studied because such material may serve as a reservoir of inoculum for further spread of oak wilt. This report presents the results of these studies.

Ten dying red oaks were felled in the summer of 1952. Small branches were cut off and piled on the forest floor. In addition, some twigs were stored at various relative humidities in desiccators in the laboratory at 24° C. Some larger branches and trunk billets were debarked and piled, whereas on others the bark was left intact. Isolations from this material and from the sawdust obtained from the sawing, were attempted at the time of treatment and at intervals after treatment.

Isolations were also attempted from the large roots within 6 feet of stumps of diseased trees treated with various silvicides. Ammate (1 lb. per gallon of water), sodium arsenite (4 lbs. arsenic trioxide and excess sodium hydroxide per gallon of water), and 2,4,5-T (4 per cent by volume in kerosene) were applied with a brush to saturate root collar frills.

Successful isolations were obtained most frequently from the twig and root samples. As the time interval between treatment and isolation lengthened, the development of secondary organisms became more frequent. Since the number of trees tested was small, and since results were variable, percentages are not comparable. The most significant result is the maximum period of successful isolation indicating the survival ability of *E. fagacearum* in the different treatments. They were: twigs (in the forest) 3 weeks; twigs (in desiccators) 11 weeks; peeled billets 1½ weeks; non-peeled billets 44 weeks; sawdust, at time of sawing only; roots of ammate-treated stumps 44 weeks, of arsenite-treated stumps 22 weeks, and of 2,4,5-T treated stumps 15 weeks.

It would appear, on the basis of these preliminary results, that the oak wilt fungus is not very long-lived in twigs and small branches. However, the experiment was performed during a very dry summer. It is possible that the fungus may live longer in such material under the weather conditions of other seasons, since Bretz and Morison[3] found that the fungus survived longer in twigs stored at low temperatures.

The fungus survived only a short time in peeled trunk billets in dry, summer weather. Yet the ability of the fungus to sporulate abundantly on peeled billets and on the underside of exposed bark in moist fall weather three weeks after peeling, as reported by Fergus,[4] indicates that under such conditions the fungus may survive for longer periods.

These results show that if the bark is left intact, billets and logs may serve as a reservoir for spread of the fungus since *E. fagacearum* may survive for months in such material. In addition, the possibility exists that mycelial mats may form on such material.[4]

Successful isolations from the roots of trees treated with silvicides indicate that they were ineffective in killing the fungus in the roots. Either the silvicides did not penetrate the roots, or the amount which did penetrate was not toxic to the fungus.

The oak wilt fungus can survive in the roots of cut trees for at least one year. The importance of the survival of the fungus in the roots in the spread of the disease, other than through root grafts, is unknown. However, its ability to survive in the roots emphasizes the importance, in eradication, of obtaining a complete kill of the healthy trees surrounding a diseased tree or killing the fungus by application of chemicals. If such trees sprout, the possibility of the disease jumping the barrier of killed healthy trees must be considered.—Department of Botany, The Pennsylvania State College, State College, Pennsylvania.

Inhibition of Tobacco Mosaic Virus by an Antibiotic from an Actinomycete, Nocardia species. DAVID E. SCHLEGEL AND T. E. RAWLINS. Woodruff has described an antibiotic obtained from an actinomycete provisionally classified as *Nocardia formica.*[1] He reported that this antibiotic significantly prolonged the life of mice infected with swine influenza virus. Two prep-

[1] Contribution No. 182 from the Department of Botany, Pennsylvania Agricultural Experiment Station. Authorized for publication November 12, 1953, as paper No. 1837 in the Journal Series. Based on a portion of a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College, in partial fulfillment of the requirements for the degree of Master of Science.

[2] The authors gratefully acknowledge the cooperation of W. C. Bramble and W. R. Byrnes of the Department of Forestry, The Pennsylvania State College.

[3] Bretz, T. W. and D. W. Morison. 1953. Effect of time and temperature on isolation of the oak wilt fungus from infected twig samples. U. S. Dept. Agr., Pl. Dis. Reptr. 37: 162-163.

[4] Fergus, C. L. 1953. Mycelial mats of the oak wilt fungus. Pa. Agr. Exp. Sta. Progress Report 100. 8 pp.

[1] Woodruff, H. B. 1953. The antibiotics of a species of Nocardia with special reference to their influence on pneumotropic virus infections. Proc. 6th Internat. Cong. Microbiol., Rome, Sept.

arations of this antibiotic #MK61 were supplied to us by Merck and Co., Inc.[2] MK61-52R2180 is an unpurified preparation supplied to us in a solution. The concentration of antibiotic in this solution has not been accurately determined. The other preparation, MK61-53R300, is a highly purified preparation supplied to us in crystalline form.

The effect of solutions of these preparations on tobacco mosaic virus multiplication in leaf discs floating on such solutions was studied. The virus in the discs was purified, and its concentration was determined spectrophotometrically. The percentage inhibition was based on the amount of virus produced in the same number of infected leaf discs floated on distilled water. The antibiotic was tested under several light environments: 1100 foot-candles and 300 foot-candles of artificial light for 12 hours each day, 25 foot-candles of diffuse daylight, and total darkness. The controls on distilled water were adjacent to the treated discs and under identical light conditions. The details of the method are described by Schlegel and Rawlins.[3,4]

The unpurified MK61-52R2180 showed no detectable injury to leaf discs at 1 ml./100 ml. and was used only at this concentration. The purified MK61-53R300 caused visible injury to leaf discs at 0.01 per cent, but no visible injury at 0.005 per cent or lower concentrations.

Quantitative data showing the inhibition of tobacco mosaic virus multiplication by solutions of MK61 are in Table 1. The data on percentage inhibition represent the average of 2 replicate experiments. Although adequate light approximately doubled the virus yield over that produced in the dark, light did not appreciably affect the percentage inhibition. The unpurified antibiotic, at the single concentration used and the purified MK61 at the concentrations of 0.001 per cent and above produced 69 to 90 per cent inhibition, regardless of light conditions. MK61 differs from certain other virus inhibitors whose action is markedly affected by light.[4,5] This effect of light on the action of virus inhibitors is at least in certain cases apparently due to the effect of light on the composition and metabolism of green leaf tissue. Since the inhibitory action of MK61 is essentially independent of light conditions, it may act primarily on virus multiplication rather than on host metabolism. This interpretation

TABLE 1.—*Effect of the antibiotic MK61 on tobacco mosaic virus multiplication under different light intensities*

Preparation	Light, foot-candles	Conc. of antibiotic	Percentage inhibition of virus multiplication	Average per cent deviation from mean
Unpurified MK61	300	1 ml./100 ml.	69	.8
	300	1 ml./100 ml.	73	5.5
	25	1 ml./100 ml.	90	7.7
Purified MK61	1100	0.001%	73	8.3
	0	0.001%	74	4.5
	1100	0.005%	86	1.9
	1100	0.001%	80	6.3
	1100	0.0001%	23	5.9

is in agreement with that of Woodruff[1] who found that MK61 is specific for certain viruses and has little effect on others. He therefore suggested that the MK61 did not inhibit animal viruses through injury of host tissue, since compounds which act primarily through toxicity to the host do not show a high degree of specificity in their antiviral spectrum.

In summary, MK61 is the most effective of the antibiotics which we have tested for inhibiting tobacco mosaic virus multiplication in floating leaf discs. Unlike a number of organic compounds, its action appears to be relatively independent of changes in host composition produced by light; its inhibitory action on virus increase may therefore be directly on virus multiplication rather than on host metabolism.—Department of Plant Pathology, University of California, Berkeley 4, California.

Soaking Treatments for the Control of Loose Smut of Barley.[1] CURT LEBEN AND D. C. ARNY. Tyner[2] has recently described tests in which the loose smuts of barley and wheat were successfully controlled by soaking seed in water or in a suspension of Spergon (tetrachloro-*p*-benzoquinone). This work is of special interest because the inciting fungi, *Ustilago nuda* (Jens.)

[2] We wish to thank the following members of the staff of Merck and Co., Inc., who have been very helpful during this work: Drs. Karl Folkers, Per K. Frolich, J. D. Garber, T. J. Webb, and H. B. Woodruff. We also wish to thank Dr. P. A. Ark of our Plant Pathology Department who gave us our first sample of Merck's MK61.

[3] Schlegel, D. E. and T. E. Rawlins. 1953. A comparison of two methods of preparing tobacco mosaic virus for spectrophotometric assay. Phytopathology 43: 89-91.

[4] Schlegel, D. E. and T. E. Rawlins. 1954. A screening test of the effect of organic compounds on production of tobacco mosaic virus. Jour. Bact. In press.

[5] Nichols, C. W. 1953. Thiouracil inhibition of tobacco mosaic virus multiplication. Phytopathology 43: 555-557.

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[2] Tyner, L. E. 1953. The control of loose smut of barley and wheat by Spergon and by soaking in water at room temperature. Phytopathology 43: 313-16.

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TABLE 1.—*The effectiveness of water and Spergon seed soaking treatments for the control of loose smut of barley*

Variety and year produced	Treatment	Greenhouse test		Field test	
		Average no. of plants[a]	Per cent smutted plants	Average no. of heads[b]	Per cent smutted heads
Mars, 1951	None	41	7.5	581	5.4
	Water soak	35	0	251	0
	Spergon soak	14	0	41	5.3[c]
Mars, 1952	None	....	....	603	6.8
	Water soak	....	....	392	0
	Spergon soak	....	....	23	0
Plains, 1951	None	49	22.0	435	14.1
	Water soak	49	0	419	0
	Spergon soak	42	0	327	0.1[c]
Plains, 1952	None	....	....	476	9.9
	Water soak	....	....	473	0
	Spergon soak	....	....	56	0
Grower's lot, 1948	None	....	....	307	5.9
	Water soak	....	....	150	0
	Spergon soak	....	....	93	0

[a] L.S.D., 0.05 = 5 plants.
[b] L.S.D., 0.05 = 72 heads. Grower's lot not included in analysis.
[c] One plant smutted.

Rostr. and *U. tritici* (Pers.) Rostr., respectively, are borne within the seed and therefore not easily eradicated. *Ascochyta pisi* Lib., a deep-seated pathogen in pea seed, has also been reported to be controlled in laboratory tests by soaking the seed in water, and indirect evidence was presented to show that antifungal antibiotics produced during the soaking period were responsible for the control.[3] The early accounts of some of the steeping treatments for the control of loose smut[4, 5, 6] suggested to us that repetition might be of value, particularly at a different location and with different varieties. In our studies, 2 tests were made with the loose smut of barley—one in the greenhouse and the other in the field. The results, although limited, substantiate those of Tyner and therefore appear worthy of a short presentation.

Naturally infected seed was used. The 1951 seed lots were residues from field plantings. This seed had been treated in the spring of 1952 with Ceresan M (7.7 per cent ethyl mercury *p*-toluene sulfonanilide) at a rate of 0.5 oz. per bu. The 1952 lots and the grower's lot were not treated with this chemical. In the steeping procedure, seed was given a 10–12 hour presoak in distilled water at 22–24° C. This was followed by a 48 hour steep in either fresh distilled water or in a 0.2 per cent Spergon SL suspension. At the end of this time, Spergon-treated seed had no pronounced odor, whereas the seed that had been soaked in water was foul; similar reactions were also obtained by Tyner. Seed was thoroughly dried at 22–24° before planting. The 1951 seed, used in both greenhouse and field tests, was treated separately for each test. In the greenhouse experiment, made during the winter of 1952-53, there were three 50-seed replicates for each treatment, and a single replicate was planted per flat. For the field test, planted in May 1953, there were 3 replicates for each treatment, with each replicate consisting of 10 gm. of seed in a 3 m. row.

The results of the tests are in Table 1. All the water treatments were effective for smut control, as were most of the Spergon treatments. The water treatment did not appear to be phytotoxic to Plains, but was somewhat injurious to Mars and to the grower's lot. On the other hand, the Spergon treatment was deleterious in all the tests. With both soaking treatments, the variety and the condition of the seed seemed to have influenced the amount of plant injury. It is noteworthy that Ceresan M applied some time before the treatments did not appear to influence the results.

—Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

[3] Wallen, V. R., and A. J. Skolko. 1953. The inactivation of antifungal antibiotics by chlorine. U. S. Dept. Agr. Pl. Dis. Reptr. 37: 421-24.

[4] Tyner, L. E. 1951. Control of loose smut of barley by chemical and physical treatments. Sci. Agr. 31: 187-92.

[5] Tyner, L. E., and R. C. Russell. 1952. Control of loose smut in barley by tetrachloro-para-benzoquinone (Spergon-SL). U. S. Dept. Agr. Pl. Dis. Reptr. 36: 180-81.

[6] Tyner, L. E., 1952. The control of loose smut of barley (*Ustilago nuda*) by Spergon-SL. (Abs.) Phytopathology 42: 476.

REPORT AND ABSTRACTS OF THE 1954 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Southern Division of the American Phytopathological Society has concluded another successful annual meeting, held in conjunction with the 51st annual convention of the Association of Southern Agricultural Workers in Dallas, Texas, on February 1, 2, and 3. Approximately 110 plant pathologists were in attendance. Two panel discussions titled "Helminthosporium Problems" and "New Developments and Problems on Nematodes in the South," respectively, were well received and acclaimed by those in attendance. In addition, a total of 29 papers was presented.

The following officers were elected at the business meeting on February 1: *President*, J. H. MILLER; *Vice-President*, T. W. GRAHAM; *Secretary-Treasurer*, G. B. LUCAS. The office of *Councilor* is administered by S. J. P. CHILTON.

JAMES A. LYLE, *Secretary-Treasurer.*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Blackrot resistance in cabbage. BAIN, DOUGLAS C. While testing seedlings of varieties of cabbage for reaction to blackrot, it was observed that some individuals failed to develop symptoms. A number of these plants were selected from variety Huguenot and selfed. Less than 5 per cent of seedlings from the selfs became diseased in seed inoculation tests. Progeny of the second inbred generation, with susceptible controls and other selections, were field-tested the fall of 1952 and spring of 1953. Inoculum was injected twice in the stems and sprayed on 3 times in both tests. Disease index of the inbred line in 1952 was 0.2, that of control was 62.4. Results of the spring test were identical except that the index of 2 control varieties was 78.5 and 82.0. Similar results were obtained with a first inbred generation selection from Early Fuji. A twice-inbred selection from Round Dutch was slightly susceptible. Progeny of the inbred line from Huguenot were crossed with diseased and healthy plants of commercial varieties. Plants from these crosses did not develop blackrot within 3 weeks after 2 injections of inoculum—all control plants succumbed within 10 days. These results indicate that there is a factor (or factors) for blackrot resistance in cabbage.

The reproduction of Tylenchorhynchus sp. from sugarcane soils on different plants. BIRCHFIELD, W. In a previous report it was indicated that an undescribed species of *Tylenchorhynchus* caused a stubby and depleted root system of sugarcane. This nematode could not be cultured on media containing various fungi but reproduced on sugarcane roots growing in sterilized soil. Examination of soil samples from the rhizosphere of several plants growing in sugarcane soils has shown the presence of this nematode in some, but absence from others. In view of this, a study was made in which certain plants were grown in sterilized soil and inoculated with a fixed number of nematodes from sugarcane. They grew for approximately 110 days after which estimates of the nematode populations were obtained. Based on the multiplication of the nematodes, Johnsongrass and rice were very suitable hosts. Pelican and Acadian varieties of soybean and Unit I Porto Rico sweet potato were fairly good hosts, while Deltapine 15 cotton, White Tuxpan field corn, and common Liqustrum failed to allow reproduction of this nematode. Easter lily was of doubtful importance.

Soil carry-over of Xanthomanas malvacearum in New Mexico. BLANK, L. M., AND R. E. HUNTER. Bacterial blight of cotton occasionally occurs in epiphytotic form in the Pecos Valley of New Mexico. Study of the role of carry-over of the causal organism from season to season in initiating epiphytotics was made over a 3-year period. Disease-free Acala seed was planted in fields where the disease was present the previous year and seedlings were inspected for blight lesions before secondary spread had occurred. Infected seedlings were found in 2 of 3 plantings in 1951, in 1 of 2 plantings in 1952, and in both of 2 plantings in 1953. The highest rate of infection was 2.4 per cent but usually the rate was much lower. Plantings ranged in size from 400 ft. of row to approximately 10 acres. In fields where sorghum or alfalfa had been grown, or the land left idle for a season following a blight-infected crop, no infected seedlings were found when blight-free seed was planted the next season. It is evident that the bacteria can survive through the winter in the soil under New Mexico conditions and infect the following season's crop.

The seed-furrow application of fungicides to control cotton stand failures. COOPER, W. E. Use of recommended cotton seed disinfestants gives satisfactory control of seed-borne diseases, but stand failures due to soil-inhabiting organisms are still rather frequent. Arndt in South Carolina has shown by laboratory studies that certain fungicides applied to the soil reduce soreshin. Eight organic fungicides were tested at Rocky Mount, North Carolina, at rates of ½ and 1 lb./A. (active ingredient). They were applied as sprays in the seed furrows over previously dropped acid-delinted, treated seed. A drought followed by a wet, cold period retarded and reduced emergence. Captan and thiram resulted in highly significant (almost 2-fold) increases in stands. Two others, Manzate 75 (manganese ethylene bis dithiocarbamate) and Crag Experimental Fungicide 5400 (alpha, alpha-trithiobis [N-dimethyl-thioformamide]) gave significant increases. There was no difference between the 2 rates of application used for these fungicides. The other 4 fungicides, Stauffer N-521 (3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione), zineb, chloronil, and Mathieson 275 (pentachlornitrobenzene) were not effective. None of the fungicides significantly affected post-emergence damping-off. The application of fungicides at rather low rates to the seed furrow appears promising in the development of controls for cotton stand failures caused by soil-inhabiting organisms.

Host-parasite relations of the gray leaf spot disease of

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tomato. DIENER, URBAN L. Histological studies showed that the pathogen (*Stemphylium solani*) invaded the tomato leaf primarily through the stomates although direct pentration also was found. The fungus entered the stomate by an infection peg or by the germ tube tip. Direct penetration occurred by infection pegs entering between epidermal cells or between the outer guard cell wall and the adjacent epidermal cell. Following penetration by infection pegs or hyphae, the development of the fungus in the leaf tissues was similar, regardless of the mode of penetration or the leaf surface invaded. Bulbous primary hyphae developed from the infection hyphae in the substomatal or other subepidermal cavities within 24 hours after inoculation. Secondary hyphae originated from the primary hyphae and ramified intercellularly through the host tissue. Extensive development of secondary hyphae was found 48 hours after inoculation. About 60 hours after inoculation, host cell protoplasts in the lesion appeared disorganized and a dense granular material was evident. Disintegration of the cells occurred in the immediate area of the invading hyphae. Cell walls in advance of the hyphae were visibly altered. Peripheral growth of the fungus appeared to cease in the later stages of infection.

Additions to our knowledge of the life cycle of taphrina coerulescens. GOODE, MONROE J., AND G. K. PARRIS. In studying the life cycle of *Taphrina coerulescens*, the causal agent of oak leaf-blister, emphasis was placed upon that portion of the organism's existence between spring dissemination of ascospores and conidia following primary infection, and the appearance of disease the following spring. It has been shown, by morphological and cytological studies, that ascospores bud to form "primary" conidia while still in the ascus. These conidia, on release from the asci germinate immediately, or germinate later in midsummer or the following spring. Primary conidia still within, or released from, asci may bud to form "secondary" conidia which function in similar manner to primary conidia. Conidia, primary as well as secondary, infect the oak leaf just as, but not before, the buds burst. Mature leaves do not become diseased; the nature of their resistance is not known. Climatic conditions prevailing at the time the oak buds burst is directly correlated with the severity of oak leaf-blister; under certain conditions a secondary cycle of disease may appear on juvenile oak leaves that develop from buds opening unseasonably during the summer. All varieties of Quercus studied, except *Q. stellata* (post oak), *Q. palustris* (pin oak), and *Q. shumardii* (shumard oak) can become severly diseased in Mississippi.

The tobacco stunt nematode in South Carolina. GRAHAM, T. W. The tobacco stunt nematode, *Tylenchorhynchus claytoni*, an ectoparasitic species, occurs generally in tobacco soils in eastern South Carolina. This nematode was present in 67 per cent of 175 soil samples collected from fields where tobacco was stunted (1951 through 1953). It was frequently present also in cotton and corn soil samples. This species proved to be parasitic on tobacco in the greenhouse and field trials. Greenhouse soil cultures were established by picking up single specimens and placing 100 or more in each pot of autoclaved soil. Populations were allowed to increase on cotton or tobacco for several months. Effects on tobacco were stunted top growth and a much retarded root system as compared with normal growth in nematode-free soil. Root symptoms were distinct from those caused by other known root parasitic nematodes. Affected roots did not show definite lesions but were shriveled and sparsely developed and did not elongate normally. Retarded growth was accompanied by a large increase in nematode populations in both greenhouse and field trials. Green weight of roots from infected plants was consistently less than that from nematode-free plants.

A late-blight-like disease on tomatoes. HARRISON, A. L. A sudden outbreak of a disease resembling late blight, caused by *Phytophthora infestans*, was observed on May 21 at Yoakum, Texas, on staked tomatoes in a shade house following a 6-day period of rainy weather. The disease was confined to the lower leaves and fruits. No lesions on fruits or leaves were found more than 18 in. above the soil. Microscopic examination of new or old lesions on either fruits or leaves failed to reveal the presence of any spores. Affected fruits and leaves kept in moist chambers had abundant mycelial growth but no fruiting bodies were observed. When pieces of affected fruits or leaves were plated on potato dextrose agar, a species of *Phytophthora* was readily isolated in pure culture. All isolates readily reproduced the disease on uninjured green tomato fruits but not on ripe tomatoes. The period of incubation ranged from 2 to 5 days. The causal agent has been tentatively identified as *Phytophthora parasitica.* Different species of *Phytophthora* have frequently been reported as causing foot rots and fruit rots but rarely leaf spots, except the late blight fungus.

Pathogenicity differences of Xanthomonas malvacearum isolates. HUNTER, R. E. AND L. M. BLANK. Cotyledon inoculation of cotton seedlings with a suspension of the bacterial blight organism, *Xanthomonas malvacearum*, makes possible accurate separation of resistant and susceptible plants. However, during the winter of 1951-1952, presumed resistant seedlings occasionally gave a susceptible reaction. In testing a number of individual isolates it was found that 2 isolates, 51-1 and 53-1, consistently infected resistant Acala seedlings. The latter isolate was compared with a composite of normal isolates in a field test in 1953. Mature leaves were spray inoculated and graded as resistant or susceptible. Ninety-six rows of Acala breeding material, possessing Stoneville 20 type resistance, were susceptible to isolate 53-1 and resistant to the composite inoculum; 6 rows of Acala 8373 (type of resistance undetermined) were resistant to both inoculum sources; and 2 rows of commercial 1517 C were susceptible to both sources of inoculum. Eighteen additional isolates, obtained from blight resistant cottons at 4 localities in New Mexico and Texas, were pathogenic to blight resistant Acala seedlings. The existence of this strain of *X. malvacearum* and the indication of its natural distribution present a theat to an Acala breeding program utilizing Stoneville 20 as the single source of resistance.

Factors influencing oospore germination in Peronospora tabacina. LUCAS, G. B. AND L. H. PERSON. Oospores of *P. tabacina* in dried tobacco leaves which had been infected with blue mold, after exposure for 4 months to weekly alternating periods in which temperatures were held at 50°F. and 68-78°F., were placed on moist soil. About 1 month later some of the oospores had sessile sporangia attached to them. After 20 months storage, some oospores still formed similar structures. Experiments showed 1) the sporangia would form on moist soil in the absence of tobacco seedlings, 2) the sporangia did not form in water or dry soil, 3) sporangia formed at 55°, 65°, and 75°F., and 4) sporangia formed in 3–4 weeks when oospores were kept on moist soil at 65°F. with alternating 12-hour periods of light and darkness. These sporangia form zoospores within 20 minutes after being placed in water. The regularity of their formation and attachment to the oospores suggests the possibility that these sporangia are the products of normal germination of the oospores of *P. tabacina.*

The longevity of Monilochaetes infuscans in different types of soil. NIELSON, L. W. The pathogen of sweet potato scurf, *Monilochaetes infuscans*, is known to persist in some infested soils for several years. It can be eliminated by crop rotation, but the relationship of soil type to persistence of the pathogen is not known. This relationship was studied by artifically infesting 3 types of soil at the Rocky Mount, North Carolina, Experiment Station with infected roots. The soil types were heavy (Coxville silt loam), medium (Norfolk fine sandy loam) and light (Jamison sandy loam). The plots were infested and planted to potatoes in 1950. Some plots in each soil type were planted with scurf-free plants in 1951, and the percentage

of diseased roots from the 3 soils was heavy, 61.9; medium, 5.2; and light, 0.0. Plots in the heavy and medium soil were again planted with healthy plants in 1953 and no diseased roots were produced. The longer survival of the pathogen in heavy and medium soils was directly correlated with the concentration of organic matter and major nutrient elements in these soils. Previous reports on the importance of organic manures in scurf development suggest that the organic matter content of the soil may be an important factor in the longevity of the pathogen.

The spread of internal cork virus into commercial Porto Rico sweet potatoes in North Carolina. NIELSEN, L. W. Internal cork was first discovered in North Carolina in 1946 in bedded roots from the 1945 crop. A survey of bedded roots from the 1946 crop was made in 1947 and the disease was found generally distributed in the Piedmont and Coastal Plain counties. Four per cent of all roots examined had corky lesions. Bedded roots from the 1947 crop of 40 farms were again examined in 1948 and 12.9 per cent had symptoms. Composite root samples of the 1952 crop were collected from community storage houses in 4 commercial areas in the Piedmont and Coastal Plain areas and those with lesions varied from 71.2 to 90.0 per cent. Disease increase was most rapid in southern and southeastern counties. Plants from infected and relatively disease-free (0.7 per cent corky) roots were grown in contiguous plots at 3 locations for 2 years. After this time, roots from both plant sources were affected equally with lesions. The pattern of spread from an area of high disease potential into healthy stock is similar to that for viruses having leaf hoppers as vectors. Sweet potatoes free of internal cork when grown on an isolated coastal island for 2 seasons remained healthy.

Resistance to Fusarium wilt and root-knot nematode in upland cotton varieties. SMITH, A. L. Twenty-five varieties of upland cotton were compared for yield, wilt resistance, and root-knot resistance at the Plant Breeding Unit, Tallassee, Alabama, on untreated soil and soil row-fumigated with 2 gallons Dowfume W85 per acre. Auburn 56 lines and H81-16, with resistance derived from Cook 307 and other Cook parentage, had the highest combined yield, root-knot, and wilt resistance. The primary commercial group of varieties, e.g., Coker 100 Wilt lines, Stonewilt, and Plains with resistance derived from Clevewilt, ranked second in combined yield and resistance to root-knot and wilt. Pandora, Empire, and D&P 3314 were each more susceptible to wilt or root-knot or lower in yield. Significant negative correlations were obtained between wilt percentage and lint yield and between root-knot index and yield. Root-knot index and wilt percentage were positively correlated thus suggesting that in addition to providing openings for entrance to the wilt organism the root-knot nematode increases susceptibility of the host in later stages of development. The data suggest that emphasis in future breeding programs should be placed upon improved nematode resistance. On untreated soil, varieties with highest combined resistance yielded 80 per cent of treated plots. At this location the root-knot nematode is predominant in comparison with other cotton infesting species.

Nub root—an apparently undescribed seedling disease of cotton as expressed in the mature plant. STEWART, ROBERT B. AND MARVIN D. WHITEHEAD. Widespread losses were noted in 1952 and 1953 in Texas. In the field the first symptom of the disease is a sudden wilting. The normal tap root is reduced and distorted or lacking and there is a tendency toward lateral root proliferation if sufficient moisture has prevailed in the upper soil layer. Cotton seedlings grown in infested soil and showing typical damping-off lesions were transplanted to sterile soil and allowed to grow to maturity. Forty-six per cent of those plants developed symptoms of nub root, whereas 4 per cent of the apparently healthy seedlings of the same lot used as a check developed symptoms. The fungi of the damping-off complex often did not penetrate beyond the endodermal layer. Under these conditions no apparent damage is done. The converse situation exists wherein the fungi ramify through the tissue, causing damping-off. It is postulated that between these extremes nub root develops. If the invading organism destroys the cambium in a given location within the young root and then the invasion is halted, the root will form a callus and begin recovery. The nature of the damage to this cambium tissue determines the direction and extent to which the nub root symptoms may develop.

A needle disease of pines caused by Dothistroma pini. THOMAS, J. E. AND G. D. LINDBERG. Austrian and ponderosa pines in central Oklahoma are often partially defoliated by a needle blight disease caused by *Dothistroma pini.* Chlorotic spots appear on infected needles during the fall and winter. These spots gradually spread, turn brown, and girdle the needles, causing the distal end to die while the base remains green. In early spring, black stromata erupt through the needle epidermis. As they mature light-colored, conidium-bearing locules are formed within these stromata. Conidia are produced in great quantities that gradually erupt through to the surface. The spores at first are hyaline, later becoming olivaceous. In germination trials the hyaline conidia did not germinate but the darker, mature spores germinated readily. By mid-August the spores are usually all disseminated and the stromatic locules empty. Intermittently from late spring until early fall the base of the infected needle dies and drops. Limited spray trials indicate that the disease can be controlled by 3 to 5 fungicide applications between May 1 and July 15. The timing and number of applications depends upon the rainfall during this active sporulating period of the fungus. Bordeaux mixture gave the best control in the present trials.

The role of plant-bed soil treatments in the control of tobacco black shank. TODD, F. A. AND G. B. LUCAS. Tobacco plant-bed soils at 3 locations known to be infested with the black shank fungus (*Phytophthora parasitica* var. *nicotianae*) were given the following treatments: 1) 1 lb. urea plus 1 lb. calcium cyanamide per sq. yd., 2) 1 lb. calcium cyanamide per sq. yd., 3) .09 lb. methyl bromide per sq. yd., or 4) no treatment. Half of each plot was seeded to a susceptible variety and half to the moderately resistant variety, Dixie Bright 101. With the susceptible variety treatments 1 and 2 reduced the incidence of black shank and treatment 3 gave complete control. With the resistant variety, no disease was observed. When plants of Dixie Bright 101 from these plots were transplanted to field plots in comparison with similar plants taken from a black shank-free bed, there was no significant difference in the incidence of black shank from any of the treatments. This indicates that if Dixie Bright 101 is used, the presence of black shank infection in the plant bed-soil is not of critical importance.

The reaction of sericea lespedeza to root-knot nematode species. WELLS, J. C., CLARENCE H. HANSON AND J. LEWIS ALLISON. Seventy selections, representing a wide range of types of sericea lespedeza (*Lespedeza cuneata*), were scored in a replicated test for their reactions to the root-knot nematode species known to occur in the Southeast: *Meloidogyne arenaria, M. hapla, M. javanica, M. incognita* and *M. incognita* var. *acrita.* The techniques described by the authors in an earlier study were followed. None of the lespedeza selections were immune or highly resistant to all the *Melidogyne* species used in this test. However, certain selections were highly resistant to individual *Meloidogyne* species and several were moderately resistant to all species in the test.

The mechanism of action of the fungicide, 2-heptadecyl-2-imidazoline. WEST, BILLY, AND FREDERICK T. WOLF. Because of the structural resemblance of 2-heptadecyl-2-imidazoline to purines and histidine, experiments were performed with *Sclerotinia fructicola* testing these substances for reversal of action of the fungicide. The medium contained 90 gm. dextrose and 14 gm. glutamic acid per liter, pH 3.4, and was designed for optimal growth of the

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organism with respect to carbon level, nitrogen level and pH. The fungicide concentration was 1:200,000. Substances were tested for reversal activity at a concentration of 1 mg./ml. Growth inhibition by 2-heptadecyl-2-imidazoline was unaffected by this concentration of histidine, hypoxanthine, adenine, caffeine, theobromine, inosine, guanosine, guanylic acid, adenosine or adenylic acid, but significant reversal was brought about by guanine or xanthine. Experiments in which the concentrations of guanine and fungicide (or xanthine and fungicide) were varied while keeping the guanine/fungicide (or xanthine/fungicide) ratio constant indicated that the inhibition is competitive. The fungicide therefore interferes with the synthesis of guanine or xanthine. Since 5-amino. 4-imidazolecarboxamide is a known purine precursor, it was searched for in filtrates of cultures grown in the presence of 2-heptadecyl-2-imidazoline. It was not found, using a method which detects as little as 5 μg./ml. Hence, the block in guanine or xanthine synthesis occurs at some other point.

Studies of a toxic agent produced by Helminthosporium victoriae. WHEELER, H. E. AND H. H. LUKE. Other investigators have shown that *H. victoriae,* when grown on complex organic media, produces a toxic agent which induces the same disease symptoms and shows the same host specificity as the pathogen itself. In the present work, a chemically defined medium was developed, upon which extremely high yields of the toxic agent were produced. Crude culture filtrates were obtained which killed seedlings of sensitive varieties of oats at dilutions of 1:1000 and which reduced root growth of such seedlings by 50 per cent at dilutions of 1:1,000,000. Seedlings of nonsensitive oat varieties were stunted but not killed by the same filtrates undiluted and were completely unaffected by filtrates diluted 1:5. Mutants, obtained from an original isolate which was highly pathogenic and produced high yields of the toxic agent, differed in ability to produce the toxic agent; these differences correlated with differences in pathogenicity. The toxic agent, tentatively designated "Victorin", was partially purified by several methods. The most efficient consisted of adsorption on charcoal, elution with acetone, evaporation of the eluate to dryness, and extraction of the residue with ether. The crude Victorin thus obtained was soluble in water, ethanol, and butanol; insoluble in ether, petroleum ether, and carbon tetrachloride.

Toxin production by the oak wilt fungus, Endoconidiophora fagacearum. WHITE, IRENE G. AND FREDERICK T. WOLF. Toxin production by *E. fagacearum* was studied in a medium containing 2.5 per cent glucose and 0.2 per cent asparagine; pH 4.0. Differences in toxin titer were found among 3 strains studied. Interrelations among growth, pH, glucose utilization and toxin production were studied comparatively in shake and stationary cultures. In shake cultures, there is more rapid growth, more rapid glucose utilization, a higher economic coefficient, rise in pH, earlier toxin production, and higher titers. Culture filtrates were fractionated by the procedure used by Dimond et al with *Ceratostomella ulmi,* and the fractions were assayed for toxic activity using leaves of *Quercus lyrata.* Two toxic components were found: an alcohol-insoluble component causes wilting of oak leaves. An alcohol-soluble component is responsible for the development of areas of necrosis. The alcohol-insoluble component is non-dialyzable, does not reduce Fehling's solution nor form an osazone, but upon acid hydrolysis Fehling's solution is reduced and an osazone is formed. This component is probably a polysaccharide. The nature of the alcohol-soluble component, which appears to be the principal toxin, is unknown. The toxin is unaffected by pH within the range 3-9, is non-volatile, is thermostable, and is absorbed from culture filtrates by charcoal.

Development of foliage symptoms associated with internal cork of sweet potato. WILLIAMS, A. S. Foliage symptoms of sweet potatoes inoculated with internal cork virus were observed in the field for 3 seasons to determine the sequence of symptoms associated with the disease. Roots of Porto Rico, Nancy Hall, and L-240 were core-graft inoculated with diseased tissue and their sprouts planted in the field. A history was kept of each leaf and in many cases specific symptoms were marked in order to follow their development throughout the growing season. Four types of symptoms were recorded: 1) chlorotic spots, 2) chlorotic vein banding, and 3) and 4) anthocyanin pigmentation around both chlorotic symptoms. These observations indicated that chlorotic areas may enlarge after initiation and remain until masked by the general chlorosis associated with senescence. Development of anthocyanin pigment correlated with low temperatures and the pigment disappeared with an increase in temperature. Porto Rico anthocyanin development showed a higher correlation with temperature than did the other varieties. Anthocyanin production also correlated with low soil fertility. Although anthocyanin rings usually formed around chlorotic areas, they were occasionally formed when chlorosis was absent. Chlorotic spotting and vein banding appear to be primary symptoms and anthocyanin pigmentation a secondary symptom associated with cooler mean temperatures.

Sclerotium rolfsii and its control with chlorobromopropene. YOUNG, P. A. AND J. A. PINCKARD. An epiphytotic of sounthern blight, caused by *Sclerotium rolfsii,* occurred near Jacksonville, Texas, in 1953. Uncontrolled by crop rotation with corn and cotton, southern blight killed 55 per cent of 1748 tomtato plants in one acre and 91 per cent of 1533 tomato plants in another acre. It also killed 89–100 per cent of the plants of 10 varieties of tomatoes in a yield test. Extensive inoculation tests for 5 years showed only weak resistance to *S. rolfsii* in any of the many varieties and species of tomatoes included. A sandy field naturally infested with *S. rolfsii* was chosen for a soil-treatment test with randomized, replicated, metal-bordered plots, each 24 sq. ft. in size. Checks were drenched with water. The treated plots were drenched with emulsions of CBP (chlorobromopropene). Southern blight killed 67 per cent of the tomato plants in the check plots. Five per cent of the tomato plants died of southern blight within 15 weeks in the plots treated with 1.14 gal. (13 lb.) of CBP in 100 gal. of water per 100 sq yds. *S. rolfsii* killed 14 per cent of the tomato plants in the plots treated with ½ the above amount of CBP per 100 sq. yds.